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DRINKING WATER CRITERIA DOCUMENT FOR LEGIONELLA

Prepared for

OFFICE OF DRINKING WATER

Prepared by

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The Office of Drinking Water, U.S. Environmental Protection Agency, has prepared a drinking water criteria document on Legionella. This document includes an extensive review of the following topics: general information and properties, occurrence in drinking water, worldwide distribution, analytical methodology, health effects in animals and humans, symptoms and mechanisms of disease production, control of legionellae in the distribution system and in plumbing systems, and risk assessment.

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LIST OF ABBREVIATIONS

ACES	N-2-acetamide-aminoethanesulfonic acid
BCYE	Buffered charcoal-yeast-extract
CFU	Colony-forming units
CYE	Charcoal-yeast-extract
DFA	Direct fluorescent antibody
DNA	Deoxyribonucleic acid
ELISA	Enzyme-linked immunosorbent assay
GI	Gastrointestinal
IC	Intracellular
ID_{50}	Dose producing infection in 50% of the test population
IFA	Indirect fluorescent antibody
i.p.	Intraperitoneal
i.v.	Intravenous
LD	Legionnaires' disease
LD_{50}	Dose lethal to 50% of recipients
LDB	Legionnaires' disease bacteria
PMNs	Polymorphonuclear neutrophils
PPA	Pittsburgh pneumonia agent
RIA	Radioimmunoassay
RNA	Ribonucleic acid
UV	Ultraviolet

ABSTRACT

Legionnaires' disease and Pontiac fever are diseases caused by a recently discovered genus of bacteria known as Legionella. The severe form of legionellosis primarily affects people whose health is compromised by underlying diseases that impair the cellular immune system, such as cancer and those involving immunosuppression. Smoking, old age, and alcohol abuse also are risk factors. Legionnaires' disease is typically an acute, severe pneumonia whereas Pontiac fever is an acute, mild, self-limited upper respiratory disease. Legionellosis is thus far a strictly human disease insofar as natural infection is concerned. It does not affect the lower animals except those experimentally inoculated. Among the common laboratory animals, guinea pigs are the most susceptible, but the disease can be induced in rats, monkeys and in other animals.

Legionnaires' disease has been diagnosed in at least 27 countries and in most, if not all, U.S. states. More than 30 epidemics have been reported. Several thousand cases of epidemic and sporadic disease are known. Six outbreaks of Pontiac fever involving ~500 cases have been reported. It is estimated that ~25,000 cases of sporadic legionellosis occur in the United States each year, but this figure is based on serologic results obtained with a demographically-limited population and may be strongly biased.

Legionellae are common in surface waters, especially thermally-altered waters, being part of the natural biological flora. Because they are relatively resistant to chlorine, they apparently bypass conventional water treatment processes and enter the distribution system. At terminal points these bacteria find niches suitable for survival and growth. Some of the niches are heat-rejection equipment, storage tanks, hot water systems, faucets, showerheads, humidifiers, and respiratory therapy equipment. High-risk cohorts of the population may be infected by contact with the Legionella from these sources. Some infections are known to occur from aerosols containing the bacteria, and it is thought that the respiratory route is the major route of infection. There are some data suggesting that infection may occur by ingestion but this has not been proven.

Legionellae have fastidious nutritive requirements in the laboratory but are able to exist and multiply in nature in nutrient-poor situations. They are stimulated by algal products. They invade and multiply intracellularly in some protozoal species in a fashion resembling their phagocytosis by lung macrophages and intracellular growth in these and other human tissue cells.

Control measures for legionellae are usually limited to patient-care institutions where epidemic or endemic nosocomial legionellosis is occurring. The most effective measures are the use of hyperchlorination to cleanse the potable water system followed by maintaining a free chlorine residual, or maintaining the hot water system at a temperature high enough to prevent growth of the bacteria. A combination of these two methods also may be used.

I. SUMMARY

Legionellosis is a newly recognized bacterial disease whose etiologic agent is a previously unknown bacterium belonging to a rapidly expanding genus and family named *Legionella* and *Legionellaceae*, respectively. The disease exists in two distinct clinical forms: 1) an acute, mild, self-limiting upper respiratory disease with generalized illness (Pontiac fever) and 2) a severe, acute pneumonia in which the lungs are the primary organ of attack [legionnaires' disease LD)].

The term legionellosis includes both legionnaires' disease and Pontiac fever. In this document, however, legionellosis refers only to LD unless otherwise specified. Only six outbreaks (~500 cases) of Pontiac fever have been reported whereas >30 outbreaks (including several thousand cases) of LD are known.

Twelve species of legionellae have either been described or publications concerning them are in press, and descriptions of 10 new species will soon be submitted for publication. Volume I of Bergey's Manual of Systematic Bacteriology (Brenner et al., 1984) should be consulted for definition and characterization of the family and species of the *Legionellaceae* plus ancillary information exclusive of that on some very recently described species.

Legionella pneumophila, the cause of the index epidemic of 1976 in Philadelphia, PA, is still the most important cause of LD. By direct fluorescent antibody (DFA) testing, from 63-73% of all positive tests for *Legionella* are positive for *L. pneumophila* serogroup 1 and a higher percentage (81-95%) for *L. pneumophila* of serogroups 1 through 6.

Insofar as natural infections are concerned, legionellae are still strictly human pathogens. Like human populations, serologic studies of apparently normal domestic animals reveal titers for various species and serogroups of Legionella. The significance of these is questionable because infections have not been reported and isolations of legionellae have not been documented.

Among laboratory animals, guinea pigs and chick embryos are highly susceptible to experimental infection followed by gerbils, rats, marmosets, monkeys, mice, hamsters and rabbits. Birds, however, are very resistant.

There are only minor differences in the disease caused by various species and serogroups of legionellae. Also, environmental and clinical strains appear to be equally pathogenic.

The legionellae contain a somewhat atypical endotoxin corresponding to thesomatic or serogroup-specific antigens of the bacteria. They also produce a lethal acid-soluble protein toxin, a cytotoxin, a hemolysin and various proteolytic enzymes that may be involved in the pathogenesis of LD.

Legionellosis has been diagnosed in ~26 countries and in all states of the United States with the possible exception of Alaska. Case rates in the United States varied from 0.11-0.35/100,000 population for the years 1976 through 1981. On the basis of the apparent incidence of sporadic LD (12 cases/100,000), it was estimated that ~25,000 cases/year may occur in the United States. The largest number of cases was reported in 1982 and 1983, perhaps reflecting greater awareness of the disease and greater availability of diagnostic materials.

This estimate was derived from a serologic survey of a demographically limited population group and may not be representative of the United States as a whole. In addition, the estimate has suffered from the inherent difficulties of extrapolating serologic findings to indicate cases of legionellosis. Furthermore, the survey included tests for only one species of *Legionella*, *L*. *pneumophila*.

Legionellae have all the characteristics of opportunistic rather than primary pathogens, being ubiquitous in the environment but producing relatively few cases of disease. Analysis of risk factors for human infection indicate clearly that persons whose cellular immunity is compromised by cancer, immunosuppressive drugs, organ transplantation, etc., have substantially increased risk of infection. Healthy persons appear to be quite resistant to infection by the legionellae.

Legionellae are common in surface waters, especially thermally-altered waters. From this source they may enter concentrators and amplifiers such as cooling towers and other heat-rejection equipment as well as colonizing internal plumbing fixtures. Several of the ~30 outbreaks of LD that have been studied have either been associated with heat-rejection equipment (cooling towers or evaporative condensers) or such a relationship was likely.

In the environment, legionellae are frequently associated with algae and higher plants. It appears that the nutritional requirements of LDB may be satisfied by algal products because the two species can be cocultured in the laboratory in media that support LDB growth only in the presence of photosynthesizing algae.

Legionellae can invade and multiply intracellularly in vacuoles in some species of amoebae and in Tetrahymena. This is reminiscent of the invasion of guinea pig and human macrophages by Legionella. Thus, LDB have a commensal or symbiotic relationship to algae and a parasitic one to some protozoa, but neither interaction is well understood.

In 1980, British investigators first demonstrated that potable water systems contained legionellae. They suggested that these systems were a probable source of infection for debilitated and immunocompromised patients. This observation has been confirmed many times, and there is ample evidence to indicate that a substantial risk exists. Concentration of LDB in cul-de-sacs, bypass piping, and especially in hot water tanks, with subsequent deposition in faucets, showerheads and humidifiers, creates a hazardous situation for susceptible hosts. Legionellae are presumably aerosolized from the colonized fittings during showering and drawing water.

The collection of legionellae from their natural habitats usually requires concentration by centrifugation or by filtration before culturing or injecting the material into animals for recovery of the organisms. Selective culture media that have been developed recently make it possible to minimize the use of animals and to culture the inoculum directly unless the sample has a high ratio of non-Legionella to Legionella.

The two major serologic tests used in the diagnosis of legionellosis are the IFA test to measure antibody and the DFA test to detect the bacterial cellular antigens. Several other serologic tests with various degrees of sensitivity and specificity have been devised.

No suitable surrogate organisms for *Legionella* have been identified in water habitats. The association of legionellae with algae and protozoa appears to be more applicable to raw surface waters than to potable water distribution systems. There is no acceptable biological or chemical indicator for legionellae in water at this time.

Because legionellosis in humans is primarily a pulmonary disease, experimental studies in animals using the respiratory route of infection appear to be the best model. The disease in guinea pigs infected by inhalation or in rats infected by intratracheal injection closely resembles that in humans. Somewhat $<10^5$ injected or inhaled, retained cells of virulent strains of *L. pneumophila* constitute the LD_{50} for these animals. Monkeys and marmosets also can be infected by the aerosol route, but the disease is usually milder than in guinea pigs and more like that seen in rats. Gerbils and guinea pigs are very susceptible to infection by the i.p. route.

Quantitative data on infectivity of *L. pneumophila* for humans has not been reported. Data derived from guinea pigs indicated that retained aerosol doses as low as 10 CFU constituted an ID_{50}. Doses producing LD_{50} effects ranged from $3.2x10^2$ to $1.4x10^5$ CFU depending upon the strain of *L. pneumophila* and a variety of other factors. In ingestion experiments with guinea pigs, 10^3 to 10^8 CFU *L. pneumophila* added to drinking water produced fever and mild systemic disease in 52% of the animals tested. A dose-response pattern was not reported.

The disease process in the lungs of susceptible animals is characterized by multiplication of the legionellae in recruited macrophages with destruction of the macrophages and the release of toxic products. The integrity of the membranes of the alveoli is destroyed, and serum proteins, together with PMNs, macrophages, bacteria and debris, fill the air sacs producing hypoxia and impairing respiratory function.

In epidemic human legionellosis, attack rates have varied from 0.3-13% for LD and from 95-100% for Pontiac fever. The incubation period for LD is usually given as 2-10 days (mean 5 days); for Pontiac fever, the median time is ~36 hours. The duration of illness during fatal pneumonia caused by LDB may be as short as 2 days and as long as 21 days with an average of 8 days. Pontiac fever is usually mild and lasts 2-5 days with no mortality.

The symptoms of LD are those frequently associated with pneumonia from other causes, but fever is usually higher, diarrhea occurs more often, and confusion and associated neurologic symptoms are more likely to be seen. Cough is usually a prominent symptom but is generally not productive of sputum.

Radiographically, Legionella pneumonia in nonimmunosuppressed patients presents itself as acute lung infiltrates. It is a fibrinopurulent bronchopneumonia that may be lobular or lobar with a 10-20% mortality if not treated, and a 0-11% mortality if treated. Most clinicians think that LD cannot be easily distinguished by clinical and laboratory characteristics from pneumonia caused by other agents. Pontiac fever resembles a mild form of LD except for the virtual absence of upper and lower respiratory tract disease.

In LD, acute alveolitis and bronchiolitis are histologically the most important pulmonary lesions. The alveolar exudate consists of a mixture of macrophages, PMNs, erythrocytes and proteinaceous material. Lysis of the cells of the exudate is common. Microscopic abscesses and coagulation necrosis of the lung tissue are observed in some cases. Large numbers of legionellae cells are seen in the exudate when appropriately stained. Ultramicroscopically, the bacteria are seen within the phagosomes of the macrophages and appear to be dividing by a nonseptate pinching process.

Extrapulmonary effects have been noted in LD, but the pathology of other organs or tissues appears to be related more to disseminated toxic effects from the pulmonary disease than to direct action of the bacteria in these sites. Chronic sequelae occur, but most patients have recovered from LD without serious pulmonary pathology.

The exact mechanism of transmission of legionellosis from putative or known sources to humans has defied definition because there are no illustrative studies with human volunteers. Critical aerobiology studies also have not been done. Quantitative data on the size and characteristics of an infective dose are not available for humans. The evidence strongly favors an aerosol route of infection for both LD and Pontiac fever, but a GI path is a possibility, at least for LD with its longer incubation period. Legionellae are so common in the environment that it is unlikely that anyone escapes contact with them. This contact may result in some degree of humoral immunity as expressed by rising antibody titers and the lack of evidence for transfer of infection from LD patients to contacts. Important risk factors for contracting Legionella infection are underlying diseases, especially those involving immunosuppression. Age, alcohol abuse, surgery, diabetes and kidney disease also are risk factors.

Immunity in LD encompasses both humoral and cell-mediated types but the latter appears to be most important. The legionellae are facultative intracellular parasites. When they are phagocytized by macrophages in the lungs, some of the bacteria are killed but the remainder multiply intracellularly at a rate equal to that in the best culture media. Eventually the macrophages are killed and the bacteria and their toxic products are released to produce additional tissue injury.

Approximately 122-136 million people in the United States are supplied surface water by community public water systems and are, therefore, potentially exposed to legionellae, if these bacteria survive the treatment process. Because hospitalized people are generally the sickest, they are at the greatest risk of infection. It was estimated that there are 9500 cases of nosocomial LD pneumonia/year in the United States. If 10% (undocumented) of these are fatal, the resulting 950 fatal cases would agree with the number calculated by applying the 3.8% fatality rate of Cohen et al. (1979) to the estimated 25,000 total annual cases of LD in the United States.

Legionellae's thermoduric characteristic is causally related to their ability to survive and multiply in thermally-polluted surface waters and in potable hot water systems. They have been isolated from hot and cold water systems in many patient-care institutions, frequently by direct plating on suitable media with or without the addition of selective agents. When legionellae are present in small numbers, concentration by filtration or centrifugation may be necessary before plating. Examination of surface water samples may require concentration and animal injection for recovery of the organisms because of the high ratio of nonlegionellae to legionellae bacteria.

If surface water supplies are a major source of the legionellae that produce sporadic and nosocomial cases of LD, what control measures are feasible? First, treated water supplies may be recontaminated after treatment and before distribution. This should be avoided by dispensing with open reservoirs or other storage facilities that are unprotected. Pure drinking water may be contaminated during distribution by broken or corroded pipes, back siphonage, cross connections, storage tanks, etc. In new facilities the water may be contaminated by failure to cleanse and disinfect the plumbing system. Several serious outbreaks of LD have occurred in newly opened buildings. Within buildings, showers, faucets and respiratory therapy equipment may be colonized by legionellae. Hot water tanks and tanks that are temporarily out of service but that are holding stagnant water are prime foci for concentration and amplification of legionellae. The two major techniques for disinfecting contaminated plumbing equipment are hyperchlorination and raising the temperature of the hot water to 55-60°C permanently or to higher temperatures (~70°C) intermittently. Hyperchlorination is not always successful with hot water systems because of the difficulty of controlling the free chlorine residual.

Hot water and other storage tanks should be drained, flushed and disinfected periodically and sludge should be removed. Above all, rubber washers and gaskets should be removed from taps and showers as well as removing sieves and other inserts that restrict the flow of water and offer a habitat for colonization by legionellae.

No standard procedure exists for disinfection of potable water to eliminate legionellae. All management techniques used thus far have been tailored to satisfy specific problems.

II. GENERAL INFORMATION AND PROPERTIES

Extent and Significance of the Problem

The term "legionellosis" encompasses both LD and Pontiac fever, i.e., diseases caused by members of the genus of bacteria known as *Legionella*. Legionnaires' disease was the name given to the acute lower respiratory disease (pneumonia) that affected the Philadelphia legionnaires in 1976. Clinical criteria for a case of LD as originally described (Fraser et al., 1977) required that a person have an illness characterized by cough and fever (temperature of 38.9°C or higher) or any fever and chest X-ray evidence of pneumonia. To be considered a case of LD, one of the following laboratory criteria must be met: a 4-fold or greater rise in IFA titer to $\geq$128, demonstration of legionellae in appropriate clinical specimens by DFA staining, or isolation of legionellae from appropriate clinical specimens (Kuritsky et al., 1984).

In 12 epidemics the attack rate in LD ranged from 0.3-13% and the case fatality ratio ranged from 8-67% (England, 1981; see Table III-5). In 11 of these 12 outbreaks the attack rate was $\leq$4%, and in 12 of 16 of the outbreaks for which the case fatality ratio was known the mortality rate was $\leq$25%.

Sporadic LD, defined as that occurring in the general population as single unassociated cases, had an attack rate in 1978 of 2.4 cases/10^6 population for 516 persons with onset (England et al., 1981). In the same study the mortality rate was 19% among 860 patients for whom data were available up to the end of September 1979. This figure may, however, be overstated because of a retrospective search for cases by review of post mortem tissue.

Kuritsky et al. (1984) compiled cases of sporadic LD occurring during the period 1970-1982 in the United States. These consisted of 2470 reported cases of which 97.1% occurred between 1977 and 1982. The fatality rate was ~20%.

Nosocomial LD is defined as either epidemic or sporadic cases contracted within hospitals or other patient-care institutions rather than in the community. In one of the first studies of nosocomial sporadic LD, the mortality rate among 71 patients was 31% (England and Fraser, 1981). In the first report on epidemic nosocomial LD from the Wadsworth Medical Center (VA hospital) in Los Angeles, 49 cases were identified, 15 (31%) of which were fatal (Haley et al., 1979). In the same hospital a prospective survey of 1658 consecutive hospital admissions revealed seven cases of LD, giving an attack rate of 0.5% for all new admissions. Legionnaires' disease accounted for 11.6% of all nosocomial pneumonias in a Burlington, VT, hospital in 1978 (Gerber et al., 1981). In the Pittsburgh VA Hospital, L. pneumophila was the cause of ~18% of the nosocomial pneumonias. Kirby et al. (1980) reported case fatality ratios for 64 patients with nosocomially acquired LD. Patients receiving erythromycin therapy had a mortality rate of 13%; those not receiving the drug had a rate of 55%. The overall mortality rate was 25%.

In more recent years, attack rates and mortality rates have varied widely from one institution to another they are, related to factors such as the nature and state of the patient's illness, therapy employed, age, sex, physical location within the institution, and the degree of exposure to the institution's water supply.

Legionnaires' disease in the United States is a reportable disease, and the number of cases reported ranges from 235 in 1976 to 704 in 1983 for a total of 4224 cases over a span of 8 years (see Table III-2). These figures probably seriously underestimate the incidence of the disease because an unknown number of cases are not diagnosed as LD even when pneumonia is present and larger numbers of milder nonpneumonic LD may occur. One widely accepted estimate is that of 25,000 cases per year in the United States (Fraser, 1980).

In contrast to LD, Pontiac fever is an acute febrile illness characterized by fever, headache, myalgia and malaise. Unlike LD it has a very short incubation period (5-66 hours) and a high attack rate (95-100%). Mild respiratory symptoms are common, but significant upper or lower respiratory disease does not occur (Kaufmann et al., 1981). Some evidence suggests that Pontiac fever may be an expression of hypersensitivity to legionellae or their products, or hypersensitivity combined with a very mild infection -- similar to "humidifier fever" (Rowbotham, 1984).

The incidence of Pontiac fever must be derived from the small number (6) of reported outbreaks that include a total of ~500 cases. Because Pontiac fever is a mild disease the is frequently not reported, it is not possible to estimate the number of sporadic cases that may occur. Fundamental studies on the mechanism of causation of Pontiac fever should have a high research priority.

Members of the genus Legionella (legionellae) appear to be opportunistic rather than primary pathogens. This conclusion is based on 1) the observation of high susceptibility to infection of patients with serious health problems coupled with the high resistance of normal persons; 2) the relatively high doses required to produce infection in susceptible animals; 3) the prevalence of the bacteria in the environment suggesting frequent contact with humans but associated with a relatively low rate of infection; and 4) the very low degree of communicability based on the absence of secondary cases of the disease. One point of view is that the health of all hospitalized patients is compromised to a greater or lesser extent and, therefore, their susceptibility to infection by opportunistic pathogens is increased.

Although legionellosis includes both LD and Pontiac fever, in this document legionellosis refers only to LD unless Pontiac fever is specifically identified.

The history of Legionella and legionellosis is obscure but almost certainly lies in the distant past. It would be naive to think that it began with the 1976 outbreak of disease in Philadelphia (Fraser et al., 1977), with the retrospectively diagnosed outbreaks in 1957, 1965, 1968, 1973 and 1974, or even with the earliest extant isolate of Legionella from a patient in 1943. If legionellae have been present in the biosphere and have been causing disease for an indefinite period, why was this fact not discovered earlier? The most probable answer is 4-fold: 1) pathologists examining lung sections of victims of Legionella pneumonia did not see the bacteria because they were using inappropriate stains; 2) bacteriologists did not isolate Legionella from lung tissue because they were using inappropriate media; 3) the scientific community and the public sector were not motivated to apply the necessary expertise and resources to find the cause of unexplained human pneumonia until an explosive outbreak of severe disease occurred in Philadelphia in 1976 -- thus, the victims of undiagnosed pneumonias were relegated to the category of "pneumonia of unknown etiology, probably viral"; and (4) concentrators and amplifiers of legionellae such as cooling towers, evaporative condensers, humidifiers and respiratory therapy equipment have been used more extensively in recent years.

The explosive outbreak of pneumonia in legionnaires attending a convention in Philadelphia in July 1976 alerted the medical community to an important human health problem and led, several months later, to the discovery and isolation of L. pneumophila, the etiologic agent of LD (Fraser et al., 1977; McDade et al., 1977). Dr. Joseph McDade was one of many scientists at the Centers for Disease Control (CDC) who were attempting to isolate the etiologic agent. Being a rickettsiologist, he inoculated lung tissue from fatal human cases into guinea pigs and passed spleen tissue from the infected guinea pigs into embryonated eggs. In the yolk sacs of the embryos, he saw organisms larger than Rickettsia and, at first, dismissed these as being contaminants from the original lung tissue or from the guinea pigs. In December of 1976, McDade reviewed his slides and was impressed with the numbers of small bacteria-like organisms seen in smears from several different guinea pigs and eggs. With an IFA technique using serum from patients with confirmed cases of LD and antihuman globulin labeled with fluorescein as an indicator, he showed that the patients' serum contained antibodies complementary to the antigens of the smear organisms. On January 18, 1977, it was announced that the cause of LD probably had been found.

An important factor relevant to the incidence of legionellosis is the rapid increase in the use of large-scale heat-rejection systems such as industrial and institutional cooling towers. Several large outbreaks of legionellosis have been epidemiologically associated with these systems (Glick et al., 1978; Cordes et al., 1980a; Dondero et al., 1980; Band et al., 1981). The large Philadelphia outbreak of LD could not be associated with such a system. Nosocomial infections have been associated with heat exchange systems, showers, nebulizers and whirlpools, especially those devices that aerosolize warm water. Thus, instruments designed to increase human comfort and to promote healing have introduced an unexpected risk factor of disease.

The incidence of legionellosis associated with domestic plumbing and air-conditioning equipment is not known. Because sporadic communityacquired legionellosis occurs throughout the year (England et al., 1981), a high index of suspicion must be directed to potential disseminators such as humidifiers, air-conditioners, showers, whirlpools, nebulizers and fountains (especially in enclosed malls). Equipment utilizing warm water not containing a disinfectant residual poses the greatest risk.

Legionellosis occurs worldwide. Experience indicates that Legionella species can be isolated from fresh surface waters almost anywhere one cares to look for them (Fliermans et al., 1981a). They are part of the natural biota of surface and, perhaps, subterranean waters. They are not found in marine environments except when deposited in estuaries by freshwater inflows (Fliermans, 1983). Also they have not been isolated from soil except for moist soil in association with bodies of freshwater.

Taxonomy, Nomenclature and Description of Species of Legionella

In 1943 while attempting to find the cause of Fort Bragg fever, Tatlock (1944) demonstrated in impression smears of guinea pig tissue and of egg yolk sacs poorly staining gram-negative rods apparently originating from the blood of a patient with an unusual febrile disease called "pretibial fever." The organism (TATLOCK) was described as "Rickettsia-like." It was not isolated on nonliving media, but infected guinea pig tissues were preserved for future study.

In 1947 a researcher inoculated the blood of a patient ill with fever of unknown origin into guinea pigs and demonstrated another "Rickettsia-like" organism (OLDA) that he was unable to culture (Jackson et al., 1952).

In 1959 Bozeman described two "Rickettsia-like" organisms (WIGA and HEBA) growing in embryonated eggs that had been inoculated with tissue from guinea pigs infected with either lung tissue from a fatal case of human pneumonia or with blood from a suspected case of pityriasis rosea (Bozeman et al., 1968). The organism did not grow on artificial media.

After the discovery of the cause of LD (McDade et al., 1977) it was necessary to find a way to isolate the organism from tissue. Many workers in diverse laboratories using a large variety of media and techniques had been unsuccessful in obtaining growth on artificial media. Shortly after McDade's discovery, Dr. Robert Weaver inoculated hen's egg yolk sacs infected with the LDB onto 17 different bacteriologic media (Feeley et al., 1978). Only one of these, Mueller-Hinton (MH) agar supplemented with 1% hemoglobin and 1% IsoVitaleX (MH-IH), supported growth of the organisms.

This is a medium that is seldom used for culturing bacteria other than the gonococcus or the meningococcus. Now the causative agent could be studied in detail and Koch's postulates fulfilled, at least in guinea pigs and embryonated eggs. Subsequently, it was discovered that the late Dr. Morris Dumoff had submitted to CDC for identification a culture that proved to be L. pneumophila. This had occurred before Weaver's isolation, and Dumoff had recovered the organisms from commercially prepared, enriched chocolate agar similar to that used by Weaver (Dumoff, 1979).

Feeley et al. (1979) undertook a detailed study of the nutritional requirements of L. pneumophila because growth on the above media required large inocula and was quite slow. They discovered that L. pneumophila required both the amino acid L-cysteine and an iron salt for isolation and growth. Growth on the supplemented MH agar occurred because these nutrients were present. They developed a superior CYE agar base supplemented with L-cysteine-HCl and a ferric salt (soluble ferric pyrophosphate). The pH for consistent good growth of Legionella is more critical than for most bacteria, being in the range of 6.85-6.95. The medium was improved still further (Pasculle et al., 1980) by the addition to the CYE agar of 10 g/ℓ of ACES buffer that served to stabilize the pH within the appropriate range. This medium is designated as agar.

McDade et al. (1979) isolated on artificial media the bacterium OLDA described by Jackson et al. (1952) and WIGA described by Bozeman et al. (1968). OLDA proved to be indistinguishable from L. pneumophila whereas WIGA was found to be genetically unrelated. WIGA was later described as a new species, Legionella bozemanii (Brenner et al., 1980).

Two strains, NY 23 isolated from cooling tower water and Tex-KL isolated from a post mortem lung specimen, constitute a third named species, *Legionella dumoffii* (Brenner et al., 1980).

Hebert et al. (1980a) inoculated onto artificial media the egg yolk sac suspensions preserved by Tatlock since 1943 and by Bozeman since 1959. They isolated from the former on CYE agar a strain designated TATLOCK and from the latter a strain designated HEBA. The two isolates were identical but differed from the previously described *Legionella* spp. in some phenotypic aspects and were unrelated genetically. Soon it was discovered that the "Pittsburgh pneumonia agent" isolated by Pasculle et al. (1980) from human lung tissue was, like HEBA and TATLOCK, phenotypically similar to *L. pneumophila* but was genetically identical to the TATLOCK bacterium (Hebert et al., 1980b).

Hebert et al. (1980c) compared the strains TATLOCK, HEBA and the Pittsburgh pneumonia agent (PPA) and found them to constitute a new species, *Legionella micdadei*. Pasculle et al. (1980) named these strains *Legionella pittsburghensis*.

Legionella gormanii was described as a new species by Morris et al. (1980). It was isolated from creek bank soil in 1978 by G.W. Gorman. There is serologic evidence that it may be implicated in human disease, but this has not been proven by isolation.

Legionella longbeachae (McKinney et al., 1981) is a causative agent of human pneumonia. It has been isolated from lung tissue and transtracheal aspirates. The species consists of two serogroups (Bibb et al., 1981).

Legionella jordanis (Cherry et al., 1982) was represented by two cultures, one isolated from river water and the other from sewage. This species has a partial relationship to L. bozemanii by DFA tests but is unrelated to any of the other five species listed above. With human sera IFA tests suggested that unrecognized human infections with L. jordanis may be occurring.

A new species, Legionella wadsworthii (Edelstein et al., 1982a), was isolated from a case of human pneumonia and shown to be the etiologic agent of the disease. This organism was genetically distinct from all other legionellae but closely resembled them phenotypically.

Legionella oakridgensis, a new species, is represented by 10 strains isolated from industrial cooling tower water in Pennsylvania and in Minnesota (Orrison et al., 1983b). These cultures differed both genetically and serologically from all other species and serogroups of the legionellae. They differed from all other species in not requiring added L-cysteine for growth in serial transfer on CYE agar. These bacteria have not yet been associated with human disease, but they are potential causes of legionellosis.

Legionella sainthelensi (Campbell et al., 1984) is a new species represented by six strains isolated from freshwater in areas affected by the volcanic eruption of Mt. St. Helens in the State of Washington. These bacteria were genetically distinct from all other legionellae but closely resembled them phenotypically. Serologically, this species cross-reacted with both serogroups of *L. longbeachae* and with *L. oakridgensis*.

Twelve species of *Legionella* have either been described in the literature or publications concerning them are in press. Ten new species will soon be described (Brenner, 1984), making a total of 22 species. Nine of the 10 new species were isolated from the environment: two from potable cistern water, three from tapwater, and one from wet soil.

Some species are divided into subgroups based on serologic differences although they are genetically homologous (as shown by DNA reassociation). *L. pneumophila* now contains serogroups numbered 1-8. *L. bozemanii* contains serogroups 1 and 2, and *L. longbeachae* includes serogroups 1 and 2.

All described species are generally considered to belong to a single genus of bacteria. Some workers, however, think that genetic and phenotypic differences are sufficient to ascribe separate genera to some species (Garrity et al., 1980; Brown et al., 1981). Thus *L. micdadei*, *L. pittsburghensis* and *Tatlockia micdadei* are synonymous as are *L. bozemanii* and *Fluoribacter bozemanae*, as are *L. dumoffii* and *Fluoribacter dumoffii*, and as are *L. gormanii* and *Fluoribacter gormanii*. To prevent confusion in perusing the literature, these relationships should be kept in mind. Accumulating experience with the legionellae will ultimately allow the scientific community to reach a consensus on the classification and taxonomy of these organisms.

Volume I of Bergey's Manual of Systematic Bacteriology (Brenner et al., 1984) should be consulted for discussion of all aspects of the classification and nomenclature of the family Legionellaceae and the genus Legionella.

Characterization of the Legionella Species

The classification and nomenclature of a new group of bacteria often produces ambivalence. The genetic purist is inclined to adhere to strict phylogenetic analysis as a basis for classification whereas the microbiologist whose laboratory data are directed to practical diagnostic problems prefers a classification that is based more on phenotypic relationships. Frequently, the two viewpoints do not coincide; i.e., genetic and phenotypic analysis do not result in the same classification. A reasonable approach to this problem is that taken by Brenner et al. (1980). They state "that genetic relatedness should be the basis for identification to species level. Ideally, a genus should contain a group of genetically and phenotypically related species. When both criteria cannot be met, phenotypic relatedness should take precedence to insure that the genus designation is of practical use at the bench."

Members of the genus Legionella have been genetically defined by DNA hybridization data, by calculation of the genome size of their DNA, by the guanine plus cytosine (G+C) content of their DNA, by the oligonucleotide cataloging of their 16S ribosomal RNA, and, to some degree, by plasmid analysis (Brenner et al., 1978; Brown et al., 1981, 1982a; Garrity et al., 1980; Knudson and Mikesell, 1980; Johnson and Schalla, 1982; Ludwig and Stackebrandt, 1983). Phenotypic relationships within the genus Legionella are shown in Table II-1.

TABLE II-1

Phenotypic Characteristics of *Legionella* Species[a]

Characteristics	*L. jordanis*	*L. pneumophila*	*L. bozemanii*	*L. micdadei*	*L. dumoffii*	*L. gormanii*	*L. longbeachae*	*L. oakridgensis*	*L. wadsworthii*	*L. sainthelensi*
Growth on agar										
CYE[b]	+	+	+	+	+	+	+	+	+	+
Blood (BAB or TSA)[c]	-	-	-	-	-	-	-	-	-	-
Blue fluorescence[d] (CYE Agar)	-	-	+	-	+	+	-	-	-	-
Browning of F-G[e] or YE[f] agar	+	+	+	-	+	+	+	+	-	NR
Stains										
Gram	-	-	-	-	-	-	-	-	-	-
Acid-fast	-	-	-	-	NR	NR	NR	-	-	-
Leifson or DFA[g]	+	+	+	+	+	+	+	-	+	+
Motility	+	+	+	+	+	+	+	-	+	+
Biochemical reactions										
Oxidase	+	+ or -	-	+	-	-	+	-	-	+
Catalase	+	+	+	+	+	+	+	+	+	+
Urease	-	-	-	-	-	-	-	-	-	-
Gelatin liquefaction	+	+	+	+	+	+	+	+	+	+
$NO_3 \rightarrow NO_2$	-	-	-	-	-	-	-	-	-	-
β-Lactamase	+	+	±	-	+	+	+ or -	+	+	+
Hippurate hydrolysis	-	+	-	-	-	-	-	-	-	-

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[a] Tabular data for species other than *L. jordanis* were taken from prior publications (see Taxonomy, Nomenclature and Description of Species of *Legionella* Section).

[b] Charcoal yeast extract

[c] Blood agar base or trypticase-soy agar

[d] Long wavelength (366 nm) UV light

[e] Feeley-Gorman (Feeley et al., 1978)

[f] Yeast extract

[g] Direct fluorescent antibody or Leifson stains of flagella; flagella are polar.

NR = Not Reported

Thus, in 1979 *Legionella* was established as the type genus within the family *Legionellaceae*. The type strain of the only species, *L. pneumophila*, is Philadelphia 1 (Brenner et al., 1979).

The legionellae are small (0.3-0.9 µm x 2-20 µm), gram-negative rods that are nonacid-fast, nonencapsulated and nonsporeforming. Most species are motile by one or more polar flagella. *L. oakridgensis* was described as a nonmotile species (Orrison et al., 1983b). The legionellae do not grow on ordinary laboratory media such as blood or infusion agar, but they do grow on media such as those used to culture the gonococcus and the meningococcus when they are supplemented with L-cysteine and iron salts. All species either require, or their growth is stimulated by, L-cysteine and iron salts especially for initial isolation from animal tissues. Even on optimum solid media such as BCYE agar, the development of colonies is usually slow (2-4 days), especially from small inocula. Once colonies appear, they continue to enlarge if well isolated and may reach a diameter of 5 mm or more after several days. On CYE agar, colonies are grey, smooth, glistening and domed with a ground-glass appearance and an entire edge. On yeast-extract agar containing L-tyrosine, a brown, water-soluble pigment is produced (Baine et al., 1978). The growth of three species (*L. bozemanii*, *L. dumoffii* and *L. gormanii*) produces an intense blue-white autofluorescence when irradiated with a long wavelength (366 nm) blue light (Cordes et al., 1979; Lewallen et al., 1979).

Legionellae are aerobic or microaerophilic bacteria whose optimum growth temperature on laboratory media is in the range of 35°-37°C. They grow slowly at lower temperatures (25°C) and generally grow well up to 43°C.

Their growth may be enhanced by culture in an atmosphere of 2.5% CO_2. More important, perhaps, is the need for a humid cultural environment. Cultures on solid media should be incubated in closed containers to prevent dehydration of the medium especially since 2-7 days (or more) of incubation are required to obtain growth. Because the pH optimum is 6.85-6.95, a buffering system is desirable to help maintain the appropriate range.

Metabolically the legionellae are nonfermentative. They do not catabolize carbohydrates (Hoffman and Pine, 1982). They have a respiratory type of metabolism and catabolize amino acids as a major source of energy and of carbon (George et al., 1980). Although the study is incomplete, Hoffman and Pine (1982) presented data indicating the L. pneumophila oxidizes amino acids through the Krebs cycle as its major source of energy. There is good agreement that the following amino acids are required for growth of L. pneumophila: L-arginine, L-isoleucine, L-leucine, L-methionine, L-threonine and L-valine (George et al., 1980; Tesh and Miller, 1981). L-serine was required for 7/10 strains and L-proline for 2/10 strains used in one study (George et al., 1980); in a second study of four strains a partial requirement for L-cysteine (or L-cystine) was observed (Tesh and Miller, 1981). All 10 strains used serine and, to some extent, threonine as the sole source of carbon and energy. L-glutamic acid was reported to be stimulatory to growth (Tesh and Miller, 1981) and to be more actively metabolized than either glutamine, acetate, glucose or succinate (Weiss et al., 1980).

A major feature differentiating legionellae from most other gramnegative bacteria is the production of large amounts of branched-chain cellular fatty acids. Relative qualitative and quantitative differences in these fatty acids are useful in species identification. Flavobacteria also contain large amounts of these acids, but these bacteria are not related (as shown by DNA hybridization) to the legionellae (Brenner et al., 1978).

The ultrastructure of L. pneumophila shows electron-lucent nucleoids and well-defined ribosomes enclosed within a double envelope consisting of two three-layer "unit membranes" (Chandler et al., 1979a).

The cell envelope consists of three layers: a cytoplasmic membrane, a peptidoglycan layer constituting the cell wall, and an outer membrane (Flesher et al., 1979). Keel et al. (1979) could not demonstrate a peptidoglycan layer, although chemical analysis indicated its presence. Also they did not detect diaminopimelic (DAP) acid, which is present in the peptidoglycan structure of most gram-negative bacteria. Guerrant et al. (1979), however, detected both MESO-DAP and DD-DAP but not LL-DAP in both whole cells and cell-wall fractions of several strains of L. pneumophila. Only MESO-DAP was detected in the partially purified peptidoglycan of L. pneumophila analyzed by Amano and Williams (1983). It seems clear that the peptidoglycan layer of L. pneumophila is consonant with that expected for a gram-negative bacterium.

Sudan Black B staining of smears of legionellae often reveal dark staining bodies within the intracellular vacuoles. These inclusions are thought to contain poly-ß-hydroxybutyrate granules that are often present in gram-negative bacteria (Chandler et al., 1979b).

Host Range

Insofar as natural infections are concerned, legionellae are still strictly human pathogens. Although these bacteria are widely distributed in the environment, there are no reports of their isolation from naturally infected animals. Considerable serological evidence exists of contact, or possibly subclinical infection, of both humans and lower animals by the legionellae. Pritchard et al. (1980) examined 164 cattle and 112 pig sera, most from outbreaks of severe respiratory disease in England. All sera were negative at a 1:16 dilution by the IFA test with an antigen of serogroup 1 L. pneumophila. Because this serogroup accounts for most reported cases of human infection in Britain, they concluded that it was unlikely that cattle and pigs play a role in the epidemiology of legionellosis.

When 196 animal sera were tested for antibodies against L. pneumophila (serogroups 1-5), L. bozemanii, L. dumoffii, L. gormanii and L. micdadei by the IFA test, only two animals (1%) had a titer (1:256) against L. pneumophila serogroups (Barth et al., 1983). Conversely, 29% of the horse and 24% of the sheep sera reacted to at least one of the non-L. pneumophila spp. antigens at a titer of 1:256 (Barth et al., 1983). At a titer of 1:128, 72% of the pigs, 56% of the sheep, and 50% of the horses were reactive to at least one Legionella spp. antigen. The lowest titers occurred in the calves, perhaps because they were the youngest animals tested. Based on tests of human sera from different age groups and the prevalence of legionellosis in the older population, this appears to be a reasonable assumption.

Collins et al. (1982) used the microagglutination test to examine 2800 human and animal sera for antibodies to serogroups 1, 2, 3 and 4 of L.

pneumophila. Titers of ≥1:64 were considered positive. Equine sera yielded the most positive results (31.4%) followed by cattle (5.1%), swine (2.9%), sheep (1.9%), dogs (1.9%), goats (0.5%), humans (0.4%) and wildlife (0%). In horses the titers were age-related, with 58.1% of animals ≥13 years old being positive. Among 242 positive sera, 43.8% reacted with a single serogroup antigen whereas 56.2% reacted to multiple antigens. Because horses appeared to have greater serologic responses to legionellae than any other domestic or wild animal tested, attempts were made to infect them with L. pneumophila serogroups 1 and 3, the serogroups to which the animals had shown maximum natural antibody response (Cho et al., 1983). Both i.v. and aerosol challenge were used but clinical illness did not develop. The bacteria were not recovered from the respiratory passages or from any other tissue or organ. Serologic responses were generated, however. It was concluded that serogroups 1 and 3 of L. pneumophila had a low pathogenicity for horses. No evidence was obtained to indicate that horses are carriers of legionellae.

A serological survey of 16 different primate species including both Old and New World monkeys, as well as apes, was carried out to document antibody reactivity against the serogroup 1 antigen of L. pneumophila (Helmke et al., 1981). Sera from a variety of nonhuman primates both wild and captive were examined by the IFA test. The results indicated a wide distribution of antibodies to legionellae in primates. Most titers, however, did not reach or exceed 1:256, a standing titer that, for humans, is considered indicative of present or past infection (Wilkinson et al., 1983). A complementfixation (CF) test was used to measure antibody to L. pneumophila serogroup 1 in the sera of a number of guinea pigs, cattle, sheep, horses, rabbits and wild pigeons (Page and Lattimer, 1978). Sera from guinea pigs, cattle, sheep, horses and pigeons generally had no antibodies to the LD antigen; most rabbits had CF titers ranging from 1:4 to 1:32, suggesting an unexplained nonspecific reaction.

Protozoa including various species of amoebae and a species of Tetrahymena may act as hosts for legionellae (see Protozoa Section in Chapter III). These relationships are parasitic or symbiotic and quite different from growth stimulation described for legionellae by coculture with algal spp. (see Algae Section in Chapter III). There have been no reports of vertebrates such as fish and reptiles or of invertebrates acting as hosts for legionellae.

The evaluation of positive serologic responses in animal species in the absence of infection or colonization proved by isolation of the legionellae is a difficult problem. One cannot be certain that the criteria (titers) established for the assay of human sera for meaningful levels of antibody are applicable to other animals. Also, the biosphere surrounding other animals cannot be compared with the human biosphere. Thus, qualitatively and quantitatively, the lower animals consume different food, breathe different air, and are in physical contact with a microbiota quite different from that of humans. We do not yet know how often antigenically related nonlegionellae produce false-positive serologic tests for the legionellae in human sera. We know even less about this possibility in other animals. The increasing number of species and serogroups of legionellae multiply the chances for obtaining false-positive serologic tests if the tests are measuring responses to antigenically related nonlegionellae organisms with which the animals have had contact.

Guinea pigs and embryonated hens' eggs have been the primary host animals for the isolation of the legionellae since the organisms were first demonstrated in yolk sac smears in 1943. The first isolations from the Philadelphia outbreak were made from yolk sacs of embryos that had been inoculated with infected guinea pig spleens. Although the legionellae now are isolated from clinical specimens by direct inoculation on artificial media, the guinea pig is useful for virulence determination. It is important to measure the virulence of environmental isolates, and the guinea pig, either alone or in combination with the embryonated egg, is the best choice for this.

The relative susceptibility to infection with L. pneumophila of Hartley strain guinea pigs, Sherman rats, Mongolian gerbils, ICR mice, Syrian hamsters, New Zealand white rabbits, white leghorn chickens, coturnix quail and corneaux pigeons has been tested (Patton et al., 1979). The first three species were highly susceptible; the next three species had moderate to low susceptibility; and the birds were not susceptible. Rhesus monkeys challenged with low doses of L. pneumophila in aerosol form had only slight fever and mild histologic lung lesions. Rats were found relatively resistant to i.p. injections of strains of L. pneumophila serogroup 1 and completely resistant to intranasal inoculation of these organisms (Rolstad and Berdal, 1981). When guinea pigs and rats were challenged intratracheally with L. pneumophila, both species were infected, but rats were much more resistant to the development of pneumonia and to a fatal outcome (Winn et al., 1982).

Guinea pigs were highly susceptible to a fatal bronchopneumonia when infected by small particle aerosols (Fitzgeorge et al., 1983). A milder illness with less extensive pneumonia developed in rhesus monkeys and marmosets when they were exposed under the same conditions. Mice were resistant to infection by the aerosol route. No fatal infections occurred in guinea pigs challenged by intranasal instillation. Davis et al. (1983a) used a nose-only inhalation device to demonstrate the comparative susceptibility of guinea pigs and rats to respiratory infection with *L. pneumophila* serogroup 1. The mortality in guinea pigs was 56%; in rats, 11%.

Virulence

Species Differences Among the Legionellae. Little information is available pertinent to species differences among the legionellae that can be related to clinical presentation, histopathology or virulence. Fewer than 50 well-documented cases of pneumonia caused by non-*L. pneumophila* species have been reported (Meyer and Edelstein, 1983). Thus, there have been few opportunities to compare diseases caused by various species of *Legionella*. Myerowitz et al. (1979) reported that the clinical presentation, radiographic abnormalities and pathology in eight patients infected with the Pittsburgh pneumonia agent (*L. micdadei*) were not specific. The patients were more likely, however, to have nodular pulmonary infiltrates associated with a mild or asymptomatic presentation than did patients with *L. pneumophila* infection. *L. micdadei* infection appears to be more common in immunosuppressed patients than in other patients (Myerowitz et al., 1979; Rogers et al., 1979).

Differences in Environmental and Clinical Strains. It is difficult to document important differences in environmental and clinical strains of Legionella. Logically, there should be differences between strains of L. pneumophila that cause LD and those that cause Pontiac fever. Both diseases are caused by L. pneumophila, but the latter is an acute, but mild, self-limiting respiratory illness characterized by fever, headache, myalgia and malaise, whereas LD is an acute pneumonia (Glick et al., 1978). Pontiac fever has a short incubation period and a very high attack rate, whereas LD has a much longer incubation period and a much lower attack rate. Paradoxically, no differences have been demonstrated in strains of L. pneumophila isolated from the Pontiac outbreak and those obtained from lungs of fatal cases of LD. The Pontiac strains were equally virulent for guinea pigs exposed to aerosols of the water of an evaporative condenser that was the probable source of infection in the building where the outbreak occurred (Kaufmann et al., 1981). Guinea pigs also were infected by simple exposure to the air of the building. Pontiac fever strains were no different from other serogroup 1 strains in morphologic, cultural, serologic or physiologic tests, or in antimicrobial susceptibility, or in pathogenicity for chick embryos.

L. pneumophila lost virulence for guinea pigs and embryonated eggs when cultivated on suboptimal artificial media such as enriched Mueller-Hinton (MH) agar (McDade and Shepard, 1979). Nevertheless, these avirulent organisms produced immunity against a challenge of virulent LDB when they were used to vaccinate guinea pigs. Both virulent and avirulent LDB tended to retain the status quo when they were maintained on optimal media such as BCYE agar (Wong et al., 1981).

Virulent L. pneumophila infected normal human embryonic lung fibroblasts (Wong et al., 1980). Agar-passed avirulent strains were restored to virulence after passage in the fibroblasts and maintained their virulence for at least 12 passages on CYE agar (Wong et al., 1981). Weinbaum et al. (1983) showed that L. pneumophila cells were not phagocytized by human PMNs under the same conditions that resulted in the phagocytosis of as many as 97.5% of L. micdadei cells. Although the bacterial cells were opsonized and phagocytized, they were not killed by the PMNs.

Some authorities think that populations of legionellae inhabiting various environmental sites may be made up of both virulent and avirulent strains in varying proportions depending upon the presence or absence of plasmids, sensitivity to disinfectants, and to factors such as heat, minerals, culture media, and to the associated bacterial flora (Yee, 1984). Sampling of water habitats may, therefore, not be representative of the indigenous population. An analogy might be the well-known problem of differentiating fecal from nonfecal coliforms in water samples.

Toxins. Early studies on toxic factors of the legionellae dealt primarily with examination for the classical lipopolysaccharide (LPS) endotoxins of gram-negative bacteria. It was found that L. pneumophila did, indeed, produce gelation of Limulus amebocyte lysate but, contrary to typical endotoxins, it had a very low pyrogenicity in rabbits (Wong et al., 1979a,b). L. pneumophila also poorly induced heparin-precipitable protein and Schwartzman reactions in rabbits. In general, Legionella endotoxin is relatively low in toxicity in animals and, in addition to the above, is less lethal and causes less weight loss in animals (Wong and Feeley, 1983). The endotoxins are probably the serogroup or "somatic" antigens of the legionellae. The serogroup antigen fractions also have adjuvant activity comparable to that of the mycobacteria in Freund's complete adjuvant (Wong and Feeley, 1983).

Hedlund (1981) reported that L. pneumophila, L. micdadei, L. dumoffii, L. gormanii and L. bozemanii share a common toxic, low-molecular-weight, acid-soluble, protein antigen. This antigen is toxic and lethal to mouse macrophages. It also suppresses phagocytosis by rat and human PMNs. Animals were protected against lethal challenge doses of the toxin or of viable Legionella of different serogroups when they were previously immunized with sublethal amounts of the toxin. Thus, the acid-soluble antigen contributes to host defense by inducing cross immunity to various Legionella species, whereas the endotoxin confers serogroup specific immunity.

Culture filtrates of L. pneumophila were cytotoxic for Chinese hamster ovary cells (Friedman et al., 1980). The toxin was found to be methanol soluble, heat stable and stable through a pH range of 5-8. It is distinct from the hemolysin or protease of L. pneumophila but may be related to the common protein complex of (Hedlund, 1981; Wong and Feeley, 1983).

In addition to the above three toxins, various extracellular proteolytic enzymes may be involved in the pathogenesis of LD. Muller (1980) showed that strains of L. pneumophila degraded several proteins of the acute-phase group as well as ß-lipoprotein, the latter being active in nonspecific resistance to infection. Proteinase activity on a Na-caseinate substrate indicated that L. pneumophila was more active than any of the other species of legionellae that were tested (Berdal and Fossum, 1982). The proteinases of all species of legionellae were either identical or closely related because immunoglobulins versus *L. pneumophila* proteinase inhibited the activity of those from other species but had no effect on proteinases from bacteria of other genera. Thompson et al. (1981) demonstrated extracellular protease activity on a variety of substrates including denatured casein, gelatin, skim milk and hide powder, but not on elastin. Berdal et al. (1982) used synthetic chromogenic tri- and tetrapeptides to reveal strong chymotrypsin-like activity in concentrated extracellular supernatants of five species of *Legionella*.

Baine et al. (1979) described the hemolytic action of *L. pneumophila* on the blood of five mammalian species. They concluded that hemolysis was compatible with the production of a membrane-active exotoxin. The role of this toxin in the pathogenesis of LD has not been clarified.

Plasmids. Because plasmids may specify bacterial characteristics such as antimicrobial susceptibility and virulence, the legionellae have been examined for their presence (Knudson and Mikesell, 1980). Two of 16 strains of serogroup 2 of *L. pneumophila* contained cryptic plasmids. These plasmids were not related to mouse virulence or to other phenotypic traits.

High-molecular-weight cryptic plasmids were found in three serogroups of *L. pneumophila*, in five other species of *Legionella*, and in some unclassified *Legionella*-like bacteria (Aye et al., 1981). The presence or absence of these plasmids was not related to any phenotypic markers.

One strain each of *L. pneumophila*, *L. bozemanii* and *L. dumoffii* contained plasmids but they were absent in strains of *L. micdadei* (Mikesell et al., 1981). Three of 12 strains of *L. pneumophila* examined for plasmid DNA contained large plasmids but, again, no biologic function could be attached to their presence (Johnson and Schalla, 1982).

A study of strains of *L. pneumophila* serogroup 1 isolated from the hot water taps of two adjoining hospital buildings having the same water supply and comparable groups of patients was reported by Plouffe et al. (1984). Plasmid analysis, serology and type of colonies on BCYE agar showed that 15 of the 16 clinical cultures isolated from patients with LD were agglutinated by a single monoclonal antibody, 12/15 agglutinable strains belonged to the same colony type, and 12/15 agglutinable strains had no plasmids. Only 1/15 clinical isolates was from patients in the adjoining hospital, although the percentage of surveillance sites positive for LDB was comparable in the two buildings. These data suggest a difference in virulence of the subtypes of *L. pneumophila* found in the two buildings.

Summary

A disease later identified as legionellosis has historically been known since 1943, although the etiologic agent was not isolated and characterized until 1977. Undoubtedly, morbidity and mortality caused by *Legionella* existed in the distant past, but there is reason to think that equipment designed to increase human comfort and safety has increased the risk of infection. At the same time, the proportion of the population in the older and more susceptible age groups has increased. The techniques of modern medicine used to prolong life have created a class of patients of unusually high susceptibility to infection by opportunistic pathogens such as Legionella.

Legionellae belong to a large group of related bacteria. Twelve species of Legionella have either been described or publication of their descriptions are in press. Ten new species will soon be described and others have been recognized. L. pneumophila is the species most commonly recognized and isolated, and it is the cause of the great majority of cases of pneumonia. Legionellae are fastidious organisms in their nutritional requirements in the laboratory, but they thrive in specialized environmental habitats whose nutritional composition is still unknown.

Legionellae are small, gram-negative, nonsporeforming, short, rodlike bacteria usually requiring L-cysteine and iron salts for growth on laboratory media. They grow well on a buffered yeast-extract medium especially when it is supplemented with α-ketoglutarate. They are thermoduric and resemble other thermoduric and thermophilic bacteria in having a very high cellular content of branched-chain fatty acids. They are aerobic and microaerophilic bacteria whose optimum temperature for growth on laboratory media is 35°-37°C. In nature or in coculture with algae in the laboratory, they grow well at higher temperatures ($\geq$45°C) and remain metabolically active at temperatures as high as 63°C. Legionellae are nonfermentative. They have a respiratory type of metabolism that requires amino acids as the major source of energy and of carbon.

All species are in the genus Legionella of the family Legionellaceae as described in Volume I of Bergey's Manual of Systemic Bacteriology (Brenner, 1984). The mole percent guanine and cytosine (G+C) of the DNA of this family is 39-43, one indication of a close relationship of its members. For practical diagnostic purposes, phenotypic similarities should take precedence over genetic differences although, ideally, there should be agreement. Some workers argue that legionellae are different enough genetically to justify formation of other genera.

Legionellae are strictly human pathogens as far as natural infections are concerned. Some serologic evidence exists for possible natural infection of horses but this has not been proven. Positive serologic tests in the absence of proved infection are open to different interpretations. Laboratory animals show different degrees of susceptibility. A descending order of susceptibility might be chick embryos, guinea pigs, gerbils, rats, marmosets, monkeys, mice, hamsters and rabbits.

Significant differences in species of _Legionella_ that can be related to clinical presentation, histopathology or virulence are unusual. Some evidence exists that patients infected with _L. micdadei_ may be more likely to have nodular infiltrates and a milder or asymptomatic course of disease than those with _L. pneumophila_ infection.

Strains from patients with Pontiac fever have not been differentiated from those of patients with severe pneumonia. _L. pneumophila_ may vacillate in virulence from cultivation on media to animal (or animal cell) passage and vice versa.

A variety of toxic factors have been described for legionellae. These are a somewhat atypical endotoxin, an acid-soluble protein toxin, a cytotoxin, a hemolytic exotoxin, and various extracellular proteolytic enzymes. The role of these substances in the pathogenesis of LD has not been well defined.

Differences in environmental or clinical strains are difficult to document, but some evidence suggests that virulence may be associated with the absence of plasmids or possibly with the presence of a plasmid of a certain size. The absence of plasmids in clinical isolates in one study also was strongly associated with a specific colony type on BCYE agar and with agglutination by a monoclonal antibody. It is possible that individual hot water systems selectively harbor different subtypes or serotypes of *L. pneumophila*.

Legionellae in specific environmental habitats may vary widely in their pathogenic potential for humans, perhaps explaining why LD may or may not occur in a contaminated hospital. Research on the factors influencing infectivity and virulence of legionellae should be given high priority.

III. OCCURRENCE

Worldwide Distribution

Legionellosis is widely distributed geographically in the United States and throughout the world. The legionellae have produced disease documented by either isolation of the bacteria or by serology in at least 27 countries (Table III-1). Almost all isolations of legionellae outside the United States have been from heat-rejection equipment or from internal plumbing of buildings. Ground and surface waters are sometimes sources for cooling towers, but potable water is also frequently used. Potable water is water that is suitable for drinking or that has received treatment designed to make it so; in the context of this document, it is usually water entering the distribution system after appropriate processing at the treatment plant.

Saito et al. (1984) isolated eight strains of _L_. _pneumophila_ from soil samples of construction sites and from mud of riverbanks in Japan. Four strains of _L_. _pneumophila_ were isolated from 17 samples of pond mud and creek water in the Okayama Prefecture of Japan by Nakahama (1983). In Bulgaria, legionellae were isolated from aquatic habitats only.

As health-care professionals acquire the expertise, serologic reagents, and the media required to diagnose legionellosis efficiently, the number of cases of disease and the number of habitats of legionellae may be expected to increase rapidly.

United States Distribution

Geographic. Cases of legionellosis have been reported from every state with the possible exception of Alaska. A summary of the reported

TABLE III-1

Worldwide Distribution of Legionellosis*
(Sporadic Cases or Outbreaks)

Australia
Austria
Belgium
Bulgaria (aquatic isolates only)
Canada
Corfu
Denmark
England
Finland
France
Germany
Israel
Italy
Japan
Majorca
The Netherlands
New Zealand
Norway
Portugal
Puerto Rico
Russia
Scotland
South Africa
Spain
Sweden
Switzerland
United States

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*Source: Various publications

cases of LD in the United States for the years 1976 through 1983 is found in Table III-2. Case rates varied from 0.11 to 0.35 per 100,000 population for the years 1976 through 1981. These figures are difficult to interpret because they include (1) accumulated, retrospectively diagnosed cases; (2) both epidemic and sporadic cases; and (3) nonuniform reporting. The distribution of cases by geographic area for the years 1978-1981 is shown in Table III-3. Summation by areas for the 4 years permits a comparison of relative geographic and annual incidence that may be more valid than comparing individual states. Some states have had large outbreaks that have motivated medical personnel to greater awareness of the disease. The adequacy of facilities for diagnosis also varies from one state to another, and the population of the different geographic areas is variable. The small number of cases reported from the mountain states may or may not be significant. A summary of reported cases of legionellosis in the United States by month for the years 1976-1982 is found in Table III-4. It shows that 69% of all cases from 1976 through 1981 occurred or were reported in the 6-month period from May to October, inclusive. It is also apparent that sporadic (nosocomial) cases occurred throughout the year, since cases associated with outbreaks were not included in the figures for 1980 and 1981. This is evident also in Figure III-1.

Incidence: Outbreaks and Sporadic Cases. One estimate of the annual incidence of pneumonia of unknown etiology in the United States was 800,000 cases (Fraser, 1980). The incidence of sporadic LD was estimated to be 12 cases per 100,000 population per year, or about 25,000 cases a year in the United States, if the Seattle experience is typical of the country (Foy et al., 1979).

TABLE III-2

Summary of Reported Cases of Legionnaires' Disease United States, 1976-1983[a]

	1976	1977	1978	1979	1980	1981	1982	1983	Total
No. Cases	235	359	761	593	475	408[b]	654	704	4224
Cases/100,000 Population	0.11	0.17	0.35	0.27	0.21	0.18	0.28	NR	

[a] Centers for Disease Control, Annual Summaries, 1976-82; Reported Morbidity and Mortality in the United States, Morbidity, Mortality Weekly Reports, 1977, 25; 1978, 26; 1979, 27; 1980, 28; 1981, 29; 1982, 30; 1983, 31.

[b] Sporadic cases only

NR = Not reported

TABLE III-3

Reported Cases of Legionellosis by Geographic Division United States, 1978-1981[a]

Geographic Division	Number Cases					Population 1980 Census (millions)[b]	Cases per 100,000 Population[c]
	1978	1979	1980	1981	Total		
New England	87	58	57	71	273	12	0.47
Mid-Atlantic	118	119	118	112	467	37	0.32
E.N. Central	95	132	118	65	410	42	0.18
W.N. Central	46	50	41	46	183	17	0.24
S. Atlantic	94	96	58	38	286	37	0.16
E.S. Central	83	34	16	8	141	15	0.11
W.S. Central	53	17	14	15	99	24	0.06
Mountain	11	9	8	27	55	11	0.07
Pacific	77	63	10	26	176	32	0.03
Total	664	578	440[d]	408[d]	2090		

[a] Source: Centers for Disease Control, Annual Summaries, 1978-81; Reported Morbidity and Mortality in the United States, Morbidity, Mortality Weekly Reports, 1979, 27; 1980, 28; 1981, 29; 1982, 30.

[b] Rounded to nearest million

[c] Calculated from census figures

[d] Sporadic cases only

TABLE III-4

Summary of Reported Cases of Legionellosis by Month
United States, 1976-1982[a]

Years	Totals	Jan	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nov	Dec	Month Not Stated
1976	235[b]													
1977	359[b]													
1978	664	29	19	22	28	38	52	65	163	139	61	28	18	2
1979	578	29	26	25	37	36	49	86	99	87	42	29	32	1
1980	441[c]	29	31	33	32	37	42	54	68	57	20	23	15	
1981	408[c]	23	22	23	45	22	50	46	52	39	31	25	30	
Subtotal (1978-81)	2091	110	98	103	142	133	193	251	382	322	154	105	95	3
1982	689[b]													
1983	704[b]													
Total (1976-83)	4078													

[a] Source: Centers for Disease Control, Annual Summaries, 1976-81; Reported Morbidity and Mortality in the U.S., Morbidity, Mortality Weekly Reports, 1976, 24; 1977, 25; 1978, 26; 1979, 27; 1980, 28; 1981, 29; 1982, 30; 1983, 31.

[b] Monthly figures not available.

[c] Includes sporadic cases only.

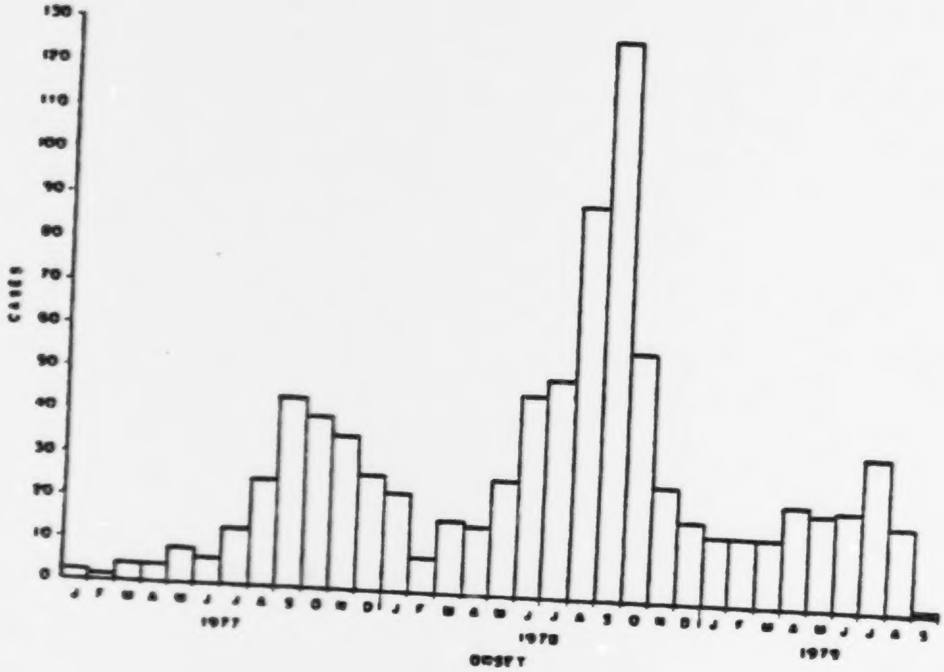

FIGURE III-1

Reported Sporadic Cases of Legionellosis
(as of September 30, 1979) by onset
United States, January 1977-September 1979

Source: England et al., 1981

Foy et al. (1979) estimated the incidence of LD in a retrospective study of stored, paired sera from 500 patients treated for pneumonia in Seattle, WA, from 1963 to 1975. The proportion of pneumonia caused by _L_. _pneumophila_ was 10/0 or 1.2 cases/10,000 person-years. All patients were members of a prepaid medical-care group. This group represented >10% of the population of metropolitan Seattle, although those under 5 and over 70 years of age were somewhat underrepresented in the sample tested. Because the Seattle study was designed to measure antibody for only one serogroup of _L_. _pneumophila_, no estimate of the prevalence of other serogroups or species was possible.

A retrospective seroepidemiologic study of LD in Iowa included the years 1972-1977 (Renner et al., 1979). The frequency of confirmed LD among the 586 cases of pneumonia was 4.1%; that of presumptive LD was 11.4%. The disease was found in most age groups but increased in frequency in males up to about age 40; in females, disease was relatively evenly distributed over all age groups. Males were 3.2 times more susceptible than females. Again, the only test antigen used was that for _L_. _pneumophila_ of serogroup 1.

The epidemiology of LD is characterized by a number of interesting parameters. Some of these are shown in Table III-5 (England, 1981) and apply to a large number of outbreaks studied in the United States and in other countries. Although sporadic cases occur throughout the year (Figure III-1), most epidemics (Figure III-2) as well as the preponderance of sporadic cases have occurred in the summer and fall months (England et al., 1981). Some outbreaks such as those in Los Angeles and Vermont have continued throughout a period of several years (Haley et al., 1979; Broome et al., 1979; Klaucke

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TABLE III-5

Epidemic Legionellosis Investigated by The Center for Disease Control, 1965-1979*

Location	Year	Dates	Cases/ Deaths	Case Fatality Ratio	Males/ Females	Setting	Source	Clinical Syndrome	Attack Rate per 100
Washington, DC	1965	July 27-Aug 31	81/14	17	57/24	Hospital	Excavation	Acute pneumonia	1.4
Pontiac, MI	1968	July 1-Aug 4	144/0	0	64/80	Health dept. building	Evaporative condenser	Acute nonpneumonic febrile illness	95
Benidorm, Spain	1973	July	8/3	38	7/1	Travelers	NR	Acute pneumonia	2.5
James River, VA	1973	July 31-Aug 1	10/0	0	10/0	Steam turbine condenser	James River	Acute nonpneumonic febrile illness	100
Philadelphia, PA	1974	Sept	11/1	9	7/4	Hotel	NR	Acute pneumonia	2.9
Philadelphia, PA	1976	July 20-Aug 16	221/34	15	166/55	Hotel	NR	Acute pneumonia	4.0
Columbus, OH	1977	July 16-Sept 9	15/NR	NA	6/9	4 hospitals	NR	Acute pneumonia	NR
Kingsport, TN	1977	Aug 1-Sept 29	33/3	10	24/9	City	NR	Acute pneumonia	NR
Vermont	1977	May-Dec	69/17	25	49/20	Community and hospital	NR	Acute pneumonia	NR
Los Angeles, CA	1977-1979	Jan-Dec	110/28	25	108/2	Hospital	NR	Acute pneumonia	0.4
Bloomington, IN	1978	Jan-Dec	39/4	10	32/7	Hotel-student union complex	NR	Acute pneumonia	0.1
Atlanta, GA	1978	July 2-7	8/0	0	6/2	Country club condenser	Evaporative	Acute pneumonia	1.5
New York, NY	1978	Aug 1-Sept 11	38/3	8	33/5	City garment district	NR	Acute pneumonia	NR
Dallas, TX	1978	Aug 18-Sept 4	19/2	11	17/2	Convention	NR	Acute pneumonia	0.5
Memphis, TN	1978	Aug 11-Sept 16	39/5	13	20/19	Hospital	Cooling tower	Acute pneumonia	0.02-0.34
Norwalk, CT	1978	Apr-Sept	26/10	39	16/10	Hospital	NR	Acute pneumonia	NR
Jamestown, NY	1979	July 2-14	7/1	14	7/0	Factory	NR	Acute pneumonia	0.7
Eau Claire, WI	1979	June 18-July 9	13/4	31	6/7	Hotel	Cooling tower	Acute pneumonia	0.2
Chicago, IL	1978-1979	Nov-July	3/2	67	2/1	Hospital	NR	Acute pneumonia	13

*Source: England, 1981

References: 1) Thacker et al., 1978; 2) Glick et al., 1978; 3) Reid et al., 1978; 4) Fraser et al., 1979; 5) Terranova et al., 1978; 6) Fraser et al., 1977; 7) Marks et al., 1979; 8) Dondero et al., 1979; 9) Broome et al., 1979; 10) Haley et al., 1979; 11) Politi et al., 1979; 12) Cordes et al., 1980a; 13) Cordes et al., 1980b; 14) England et al., 1981; 15) Dondero et al., 1980; 16) Wong et al., 1979c; 17) Shands et al., 1980; 18) Band et al., 1981; (19) Cordes et al., 1981.

NA - Not applicable; NR - Not reported

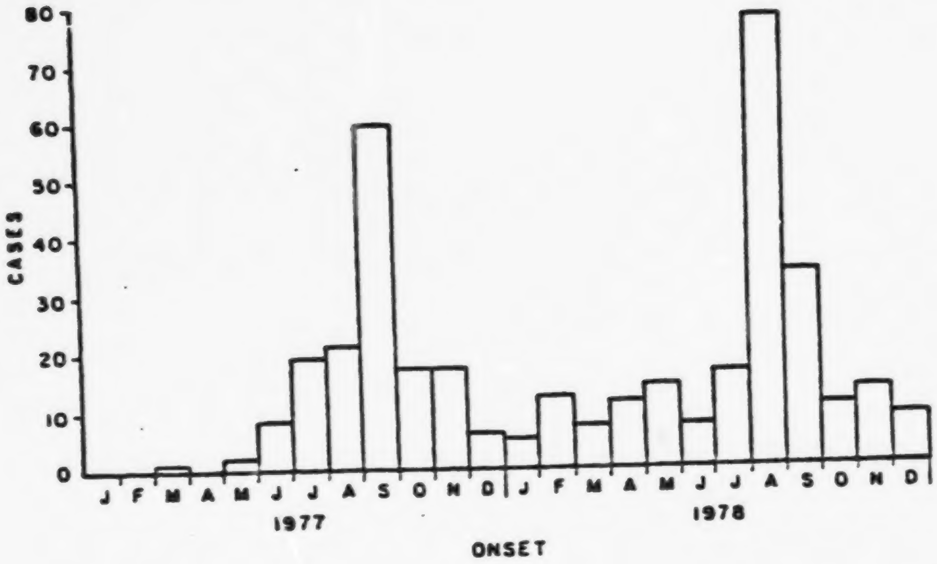

FIGURE III-2

Outbreak-Associated Cases of Legionellosis by Onset
United States, 1977-78

Source: England et al., 1981

et al., 1984). The case fatality ratio in outbreaks has varied from 0-67%. The male to female ratio of infected persons varied from 0.66 in Columbus, OH, to 54 in Los Angeles, CA, with males clearly being at greater risk of infection (Table III-5). The highest ratios of male to female patients with LD reflected the composition of the population at risk, i.e., patients in Veterans Administration hospitals (54 in Los Angeles), or in primarily male occupations, i.e., the James River, VA, outbreak (≥ 10 for Pontiac disease) in which workers were engaged in cleaning a steam turbine condenser (Fraser et al., 1979). Patients from only 2 of the 19 outbreaks listed in Table III-5 were classified as having acute nonpneumonic illness. Both of these outbreaks were classified as Pontiac disease and were characterized by very high attack rates (95 and 100%) but no fatalities. Conversely, the attack rate in patients with LD varied from about 0.1-13% with accompanying high case fatality ratios (Table III-5).

Outbreaks of LD may be explosive like that in Philadelphia in 1976 that originated in or near the Bellvue-Stratford Hotel, or they may have an intermittent point focus like that observed in the Wadsworth VA Medical Center in Los Angeles over a period of several years (Fraser et al., 1977; Haley et al., 1979). Other outbreaks have been superimposed on basic high levels of endemic legionellosis like that in Burlington, VT (Broome et al., 1979; Klaucke et al., 1984). Four of the 19 outbreaks listed in Table III-5 were epidemiologically related to either cooling towers or evaporative condensers as point sources of infection. In most of the outbreaks, a source of the legionellae was not discovered or was not proved epidemiologically to be related to the production of disease (Table III-5).

The first large study of sporadic legionellosis in the United States was done by England et al. (1981) and included all confirmed cases caused by L. pneumophila of serogroups 1 and 4 that had been reported to the Centers for Disease Control by September 30, 1979. The cases numbered 1005, of which 19% were fatal. Pneumonia documented by chest radiography was present in 98% of the 1005 cases, ~75% of which occurred from June to October. The risk of acquiring LD was greater for males than for females. Persons with debilitating diseases or those who were immunosuppressed by cancer or drugs were at increased risk of death. The attack rate calculated by England et al. (1981) for the various age groups is shown in Table III-6.

The first report of a nosocomial outbreak of LD at the Wadsworth Medical Center in Los Angeles indicated that 11% of all cases of nosocomial pneumonia were caused by L. pneumophila (serogroup 1), but less than 1% of all patients developed clinical LD (Haley et al., 1979). Clinical LD was defined as an acute illness with fever (≥39°C) in a person with symptoms or radiographic evidence of pneumonia often accompanied by prominent multisystem manifestations. The 11% incidence probably reflected the use of two additional diagnostic criteria for case definition, i.e., seroconversion and culture. Serologic surveys conducted in control populations in connection with outbreaks of LD have indicated that 4-25% of persons tested have standing IFA titers of ≥1:256 (Herwaldt and Fraser, 1982). Titers of this magnitude are considered to be indicative of past infection or contact with Legionella.

Acute LD in children appears to be a rare occurrence. In one study, paired sera from 64 acute episodes of illness in children less than 4 years

TABLE III-6

Cases of Sporadic Legionellosis and Attack Rates by Age United States, 1978[a]

Age (years)	Cases	Attack Rate[b]
0-9	5	0.2
10-19	5	0.1
20-29	33	0.9
30-39	58	2.1
40-49	74	3.4
50-59	143	6.4
60-69	109	6.2
70-79	65	6.3
≥80	11	2.3
Unknown	13	NR
Total	516	2.4

[a] Source: England et al., 1981

[b] Cases per 1 million population projected for 516 cases with onset in 1978

NR = Not reported

of age were analyzed for reactivity against serogroups 1 and 2 of L. pneumophila by the IFA test (Andersen et al., 1981). Based on serologic evidence, none of the acute illnesses appeared to be due to serogroups 1 or 2. When annual serum specimens obtained over a period of 3-5 years were compared, however, 52% of the children had seroconverted, i.e., showed a 4-fold or greater rise in titer to at least 1:128. Because most of the responses were to the antigen of serogroup 2 (Togus), an uncommon serogroup and one unassociated serologically with the acute disease, it was concluded that L. pneumophila was not a common cause of acute respiratory disease in children. The seroconversion may indicate exposure to the organism or it may illustrate cross reactions with unrelated bacteria.

Storch et al. (1979a) collected serum specimens from 1143 persons 46 years of age or older who were not acutely ill and who lived in four widely different geographic areas. Seropositivity, defined as a titer of ≥1:64, was 1.7% when the specimens were examined by the IFA test for antibody vs. L. pneumophila of serogroup 1. Thus, in this or a similar population, a titer of ≥1:64 would have a specificity of 98.3% in diagnosing an acute illness as LD. Age, sex, or geographic location were not related to the frequency of seropositivity.

Reports from state laboratories indicated a 3.1-4.5% rate of seroconversion for L. pneumophila in paired sera submitted for serologic screening in cases of undiagnosed pneumonia (Herwaldt and Fraser, 1982).

Grady and Gilfillan (1979) examined 432 consecutive pairs of sera from patients in Massachusetts. Based on both seropositivity (seroconversion or an elevated titer of at least 1:256) and on documented pneumonia, 28 (6.4%) of the patients were presumed to represent cases of LD.

An early study of the incidence of fatal nosocomial LD based on the examination of autopsy specimens of lung tissue from 263 patients with pneumonia from 24 states indicated an infection rate of 3.8% (Cohen et al., 1979). Tests were performed for legionellae of serogroup 1 of L. pneumophila only, so that the results may be considerably understated. Fay et al. (1980) used the DFA test for serogroup 1 of L. pneumophila to examine lung tissue from 224 patients in Ohio, 121 of whom died of pneumonia. L. pneumophila was demonstrated in 8 cases (6.6%). These tests suggested that 3.6% of nosocomial pneumonias in central Ohio may be caused by LDB. Meyer (1983) emphasized the unacceptability of extrapolating the data of Fay et al. (1980) to indicate that 70,000 adult deaths annually may be associated with LD. This estimate of fatal cases is, indeed, much higher than the generally accepted estimate of 25,000 total cases (fatal and nonfatal) in the United States each year. Meyer pointed out that the incidence of LD in predisposed patients during an outbreak of legionellosis was not a suitable basis for calculating national incidence of nosocomial pneumonia. Other studies have shown a similar incidence (6.7%) of previously undiagnosed LD based on examination of lung tissue (Gerber et al., 1981).

It seems clear that considerable variability exists in the level of Legionella antibody and of Legionella infection in geographically diverse areas. Because the Seattle study and most of the other studies using either the IFA or the DFA tests were designed to measure antibody for only one serogroup of L. pneumophila, no estimate of the prevalence of other serogroups or species was possible. As the number of species and serogroups increases, Legionella serology will become more complex.

Based on DFA examination of respiratory specimens submitted to the CDC during 1979, L. pneumophila was the predominant species detected (95%), although the specimens were also examined for L. bozemanii, L. micdadei, and L. dumoffii (Cherry and McKinney, 1981). Although six serogroups were tested, serogroup 1 constituted 73% of all the strains of L. pneumophila that were identified.

From June 1982 to February 1983, 72/383 (18.8%) clinical specimens received at the CDC were positive for legionellae by DFA tests (Wilkinson, 1983). Of these 72 specimens, 45 (62.5%) were positive for L. pneumophila serogroup 1 and 58 (80.6%) were positive for L. pneumophila belonging to serogroups 1 through 6. The remainder of the specimens were positive for one or another of six other species of Legionella.

The incidence of LD in the United States revealed by seroepidemiology and reviewed in this section is based primarily on the measurement of antibody by the IFA test. Although many other serologic tests have been used and others are being developed, the IFA test is the only test that has been validated with sufficient numbers of sera from well-documented cases (Wilkinson et al., 1981). This validation applies primarily to serogroup 1 of L. pneumophila. It should not be assumed that the IFA test is equally specific for other species of the legionellae.

Pontiac disease was discussed in Chapter II and will be considered in relation to LD in the following sections of this document. The two earliest epidemics, that in Pontiac, MI, and the James River outbreak, are listed in Table III-5. Six outbreaks of Pontiac fever involving approximately 500 patients have been reported, but the frequency of the disease is probably much greater because sporadic cases may not require medical attention or may not be diagnosed correctly.

Specific Occurrence

Environment.

Wastewater -- Legionellae are widely distributed in the aqueous environment in the United States and, apparently, wherever they are sought worldwide. There is no good evidence that their habitat is soil as well as water, although they have been isolated from mud or sandy, moist soil on the edge of streams (Morris et al., 1979; Cordes et al., 1980a; Fliermans, 1983).

Only two reports of isolation from wastewater has been reported (Cherry et al., 1982; Bercovier et al., 1984). A culture of *L. jordanis* was obtained from a trickling filter at a sewage disposal plant in Georgia by guinea pig inoculation. Bercovier et al. (1984) isolated *L. pneumophila* serogroup 4 and five other organisms resembling *Legionella* spp. from an oxidation pond serving as a source of irrigation water. The vast microbial load of sewage frequently results in overgrowth of any legionellae present on selective media and in premature death of injected guinea pigs. The chances of isolating legionellae from wastewaters are poor even with the use of selective media.

Ambient Water -- Fliermans et al. (1981a) surveyed 67 different lakes and streams, mostly in Southeastern United States, for the presence of legionellae. They estimated numbers of *L. pneumophila* belonging to serogroups 1 through 4 in large water samples concentrated by continuous flow centrifugation. The DFA test was used in a standardized quantitative procedure to obtain the estimate. Virtually all of 793 water samples contained bacteria that stained with one or more of the serogroup conjugates. When a concentration of $\geq 5x10^{4}/\ell$ of stainable bacteria morphologically resembling the legionellae were seen microscopically, guinea pigs were injected to isolate the suspected legionellae. Thus, 318 water samples qualified for animal testing and yielded 47 cultures of *L. pneumophila* belonging to six serogroups (conjugates for serogroups 5 and 6 had become available during the study). Serogroups 1 (63.8%) and 4 (27.7%) occurred most frequently, accounting for over 90% of all isolates. The relationship of the high- and low-range characteristics of temperature, conductivity, pH, dissolved oxygen, presence of chlorophyll a and pheophytin, and Secchi disk measurements to the frequency of isolation of *L. pneumophila* was estimated statistically. The higher temperature range of 36-70°C was the only parameter that was significantly ($p=0.0021$) associated with isolation of legionellae. In the next section of this review, further evidence of the association of legionellae with warm water is discussed. The greatest isolation efficiency (50%) noted in any parameter was observed for samples collected from habitats with a temperature range of 40-60°C. One isolate was obtained from canal water from a utility nuclear reactor having a temperature of 63°C. Isolates were obtained from samples with water temperatures of 5.7-63°C, indicating that a great range of habitats may support viability of this bacterium. A seasonality of guinea pig infection was noted in samples of water from a pond containing a relatively constant level of L. pneumophila-like bacteria throughout the year. Infection rates ranged from 10% in November and December to 69% in May, June, and July. It is not known whether or not this difference reflects variation in virulence, competitive inhibition, or stimulation by other aquatic flora, changes in host response, or chance.

People visiting the Mt. St. Helen's blast zone following the 1980 eruption became ill with a respiratory disease resembling Pontiac fever, but it was not proved that legionellae were the cause. Legionellae were, however, isolated from several of the lakes formed by the blast (Tison et al., 1982; Campbell et al., 1984). By DFA enumeration, concentrations of legionellae ranged from 1×10^4 to 2×10^7 cells/ℓ (Tison et al., 1982).

Potable Water -- In the Philadelphia outbreak in 1976, ill delegates were significantly ($p=0.01$) more likely to have drunk water in the outbreak hotel than were well delegates (Fraser et al., 1977).

The first hint that drinking water might be a habitat for legionellae appeared when Skaliy and McEachern (1979) reported that the bacteria could survive in tapwater for 364-415 days, in distilled water for 69-139 days, but in cooling tower water for only 1-2 days. Viable L. pneumophila and non-Legionella bacteria existing together in a showerhead were found to grow when the fixture was immersed in nonsterile tapwater from the fixture (Yee and Wadowsky, 1982). This combination of organisms was also recovered from a centrifuged sample of tapwater from a surgical intensive care unit. The

LDB in both samples multiplied at 32, 37 and 42°C. The non-Legionella bacteria grew at 25, 32 and 37°C.

Yu et al. (1981) cited an experiment in which a volunteer gargled and drank water containing 24,000 CFU/mℓ of LDB. The bacteria could be isolated from the oral pharynx 0.5 hour later. This experiment was motivated by the observation of 10 cases of LD after documented episodes of aspiration. Also, it was not possible to establish an epidemiologic association between LD and showering, surgery, or bronchoscopy in an epidemic situation studied by the authors. It was postulated that patients drank contaminated water and that the LDB survived for short periods in the oral pharynx following which they were aspirated into the lungs by either simple aspiration or by endotracheal manipulation for surgery or by gastroscopy or bronchoscopy.

Saravolatz et al. (1984) found the oropharynx of 5/132 (3.8%) renal transplant recipients and hemodialysis patients to be colonized with legionellae as measured by DFA staining reactions. Patients were colonized with L. dumoffii and L. micdadei, and one patient remained colonized for 6 months without developing an infection.

An interesting experiment was performed by Meenhorst et al. (1983) in which guinea pigs were infected by an aerosol of an unusual strain (Le-1) of L. pneumophila that was epidemiologically associated with six cases of nosocomial LD. The bacteria in hot (48°C) tapwater were concentrated and aerosolized to the test animals. Seven of eight animals developed pneumonia from exposure to strain Le-1; none of the two groups of control guinea pigs was affected.

While investigating a GI route of infection in guinea pigs by legionellae, Katz and Matus (1984) were able to produce fever of ≥39.4°C in 29/56 (52%) animals by inoculating virulent serogroup 1 L. pneumophila into their drinking water. A mild pneumonitis and splenitis was observed; the challenge organism was isolated from a few animals; and 14% of them seroconverted. Thus, a self-limited febrile illness was seen in some guinea pigs ingesting water inoculated with LDB.

Tison and Seidler (1983) examined drinking water at various stages of treatment in six water treatment facilities and water distribution systems. By the DFA test, sediments concentrated from all samples were positive for legionellae but no cultures were isolated. The number of cells reacting in the DFA test in raw waters was >10 times the number reacting in the distribution waters. Presumably, isolates were not obtained because the temperature of most samples was ≤13.5°C.

The French investigators (Dournon et al., 1982) described a case of legionellosis in which the patient was presumably infected by gastric lavage with tapwater found to be contaminated with L. pneumophila serogroups 1, 3, and 6. The LDB were obtained from the water and sediment of the showerheads and mixing valves. The patient developed pneumonia on the 10th day and had a strong serologic response to L. pneumophila of serogroups 3 (4096) and 6 (2048). He responded well to treatment with erythromycin.

Edelstein et al. (1982b) analyzed 85 samples of potable water from the Wadsworth VA Medical Center hospital buildings by both direct plating and by guinea pig inoculation. L. pneumophila was isolated from 33 samples by both techniques: 14 samples were positive only by direct plating; two samples only by guinea pig inoculation. Direct plating was significantly ($p \leq 0.01$) more sensitive than animal inoculation for isolating L. pneumophila from potable water.

It is worth noting that potable water entering a hospital or other building may or may not be contaminated with Legionella. No isolations of these bacteria have been reported from the extramural components of community water distribution systems. Based on indirect evidence, many investigators believe that water in the distribution system may be contaminated with legionellae although, perhaps, infrequently and with low numbers of organisms. Cross connections between cooling towers, evaporative condensers, lawn sprinkling equipment, hoses attached to faucets, etc., and the building water system are potential avenues for introduction of legionellae into drinking water.

Rainwater -- No published reports of the isolation of legionellae from rainwater have appeared, but these organisms were isolated from water of the cupped leaves of bromeliads in the El Yunque rain forest of Puerto Rico (Hazen, 1983). Turner et al. (1984) detected three species of legionellae in rainwater collected in several locations in Pennsylvania. Positive results were obtained by use of the DFA technique, but they were unable to culture any legionellae. Because positive staining of environmental specimens by the DFA test is only a presumptive indentification, their results must await confirmation by cultural isolation.

Association of Legionella with Other Biota --

Algae. A possible explanation for the widespread distribution of legionellae in nature was suggested by the observation that L. pneumophila serogroup 1 was growing in close proximity to a cyanobacterium (Fischerella spp.) in an algal-bacterial mat community at a temperature of 45°C. The alga and its associated bacteria were cultured in a mineral salts medium in the laboratory at 45°C, and L. pneumophila grew in serial transfer in association with the alga over a pH range of 6.9 to 7.6. Presumably, Legionella was utilizing extracellular organic substrates and cofactors produced by algal photosynthesis.

Berendt (1980) found that aerosols of L. pneumophila suspended in tryptose-saline solution were significantly ($p<0.01$) more stable at a relative humidity (RH) of 80% than at an RH of 30%. The experiments were repeated at 80% RH using the algal extract of Fischerella spp. as the suspending medium. L. pneumophila was significantly ($p<0.001$) more stable (viable) when aerosolized in the algal extract than in tryptose-saline (Berendt, 1981). No difference was found, however, in comparative infectivity for guinea pigs of aerosols suspended in the two media.

Pope et al. (1982) cocultured strains of L. pneumophila serogroup 1 or 4 in a mineral salts medium at 35°C with each of nine algal species including both blue-green and green algae. All of the algal species supported some multiplication of at least one of the Legionella strains showing that Legionella can use organic nutrients provided by algae for growth. The Legionella remained viable in the cultures for 60-80 hours after cessation of growth and initiated growth again when they were transferred to a new medium.

Hume and Hann (1984) studied the growth of L. pneumophila in association with green algae (Chlorophyta), observing that Legionella as well as two other bacterial species grew in microcolonies among some of the algal cells. They concluded that green algae favorably influenced the growth of Legionella but were uncertain of the mechanism. The legionellae have a kind of symbiotic or commensal relationship to many algae and, perhaps, to higher aquatic plants, but the nature of this interaction is not understood.

Protozoa. Rowbotham (1980a) was the first to observe that L. pneumophila infected free-living, ubiquitous, freshwater and soil amoebae of the genera Acanthamoeba and Naegleria. Three species of Acanthamoeba and two species of Naegleria supported the intracellular multiplication of all or some of the legionellae at 30°C. The legionellae were concentrated in the endoplasmic vacuole. Infected amoebae were fragile and easily ruptured. Rowbotham suggested that vacuoles or intact amoebae full of legionellae could be the infective particle for humans. Studies with seeded material indicated that amoebae could act as a kind of enrichment, scavenging and concentrating legionellae from water to facilitate isolation. Rowbotham (1984) also observed that various strains of legionellae have a specified host range for various species of amoebae.

Rowbotham (1980b, 1981) and Nagington and Smith (1980) postulated that Pontiac fever might be a type of humidifier fever or hypersensitivity pneumonitis caused by antigenic debris produced by the interaction of the bacteria and the amoebae. Inhalation of this material might cause a mild legionellae infection and an immunizing stimulus protecting against development of LD (pneumonia) but giving rise to a specific antibody response.

The spectrum of legionellae infectivity is not limited to the amoebae because Fields et al. (1984) found that Tetrahymena pyriformis, a ciliated protozoan, was an effective host for L. pneumophila. In sterile and distilled water the bacteria increased intracellularly in the protozoan from 1×10^2 to 5×10^4 cells/mℓ in 7 days.

Amoebal cysts may incorporate Legionella and, because they are resistant to chlorine, the cysts may provide a mechanism for transmission of the Legionella throughout a potable water system (Skinner et al., 1983).

In his most recent experiments (Rowbotham, 1983), amoebal enrichments (several different formulas were tested) were compared to direct plating as a method for isolating legionellae from either respiratory secretions or lung tissue from infected patients. Specimens were either liquefied or homogenized and added to a liquid medium that stimulated motility of the legionellae, thereby increasing the infection rate of the amoebae. Acanthamoeba polyphaga was used because it is a host for L. pneumophila of serogroups 1 through 6 as well as some other species of legionellae. It also grows on legionellae media and it is available as an axenic culture. Rowbotham examined specimens from seven cases of LD from which he isolated L. pneumophila serogroup 1 five times by amoebal enrichment and four times by direct plating. Virulent legionellae were attracted to and attacked suitable host amoebae. Apparently this represents infection rather than chance ingestion.

Bacteria. Bacteria, as well as algae and protozoa, may provide a nutritional environment conducive to the growth of legionellae. This was shown by Wadowsky and Yee (1983) in experiments with L. pneumophila serogroups 1 and Flavobacterium breve, both of which were isolated from hospital hot water tanks. L. pneumophila appeared as satellite growth around colonies of F. breve on media deficient in L-cysteine, an amino acid required by L. pneumophila. It was inferred that F. breve was supplying L-cysteine or a related compound. Future studies may identify other bacteria that contribute to the nutrition of legionellae in nutrient-poor potable water systems. Such bacteria may ultimately prove to be indicators of the presence or potential presence of legionellae in these habitats.

Internal Plumbing Systems.

Hospitals -- An article in Science in November 1980 (Marshall, 1980) discussed the problem of hospitals harboring a built-in disease source; namely, legionellae in their cooling towers. The problem is much more complex because it is now known that these bacteria frequently reside in a variety of plumbing equipment such as faucets, showerheads, hot water tanks, water storage tanks, and even in nebulizer basins.

The first proof that plumbing fixtures were a habitat for LDB was contained in a report by Tobin et al. (1980). Two renal transplant patients having occupied the same postoperative cubicle developed pneumonia from infection with L. pneumophila. Water collected from the mixing assembly of the shower baths and from the side arms, which contained brass sieves, yielded isolates of L. pneumophila of the same serogroups found in the patients. The water tanks supplying the unit and the shower fittings were disinfected and the system remained free of legionellae for at least 6 months after which the organisms were again isolated. Because the legionellae-positive showers were used infrequently, it was postulated that a "buildup" of bacteria occurred that increased the chance of infection.

In studying an outbreak of 21 cases of nosocomial LD that occurred in March 1980 throughout the Wadsworth VA Hospital, a search for a common source of infection was initiated (Shands et al., 1980). When water from cooling towers and from showers was tested, the former was negative, but 45% of the latter was positive for _L_. _pneumophila_ serogroups 1 and 4, the same serogroups isolated from patients. It was shown that changes in operating pressure within the water system dislodged sediment and scale from the pipes. On February 27, just preceding the outbreak, a pressure drop had occurred and sediment had been seen in the water. Data showed that patients and controls consumed equivalent amounts of water but patients took more showers. The study suggested that potable water transmitted LD by the aerosol route.

An outbreak of 12 cases of LD at Kingston Hospital in England resulted in extensive investigation of the sources of infection (Fisher-Hoch et al., 1981a). Although the plumbing systems of both the old and new hospitals were contaminated with LDB, all infected patients were in the new building. The new building had a cooling tower that was contaminated with _L_. _pneumophila_ serogroup 1. Legionellosis occurred, however, both before the tower was put into operation and after it was disinfected. Chlorination reduced the level of LDB in the tower but did not eliminate them. Legionellae were isolated from all hot and cold water taps in the new building. They were not found in reservoirs or in the incoming water supplies to the hospital. The cold water supply was freed of legionellae by maintaining continuous uniform levels of free chlorine (1-2 mg/ℓ). The hot water supply was decontaminated by maintaining the temperature at 55-60°C. The next year, however, another case of legionellosis occurred that was strongly associated epidemiologically with putting into operation a standby hot water tank supplying water for the patient's bath and shower. Investigation showed this tank and its sediment to be highly contaminated with the same organism (L. pneumophila serogroup 1) that infected the patient. The tank could not be decontaminated with water containing 50 mg/ℓ of chlorine, but it was freed of legionellae by maintaining a constant water temperature of 70°C for 1 hour (Fisher-Hoch et al., 1982).

Neither a contaminated cooling tower nor contaminated potable water could satisfactorily explain the original outbreak at Kingston Hospital. LD patients had not showered or bathed more frequently than noninfected patients. It was assumed that patients became infected by inhalation of aerosols from cooling tower exhausts or from those produced by taps or showers. In fact, later experiments proved that 10-μ particles generated by a spinning disc located to simulate aerosols produced from the cooling tower on the roof could, indeed, be detected inside the hospital (Fisher-Hoch et al. 1981b). Air samples were positive for the generated particles at several locations inside the top floor (lee side) and, in high concentration, near a ward window opening onto an internal well.

Colbourne et al. (1984a,b) discovered that rubber, but not plastic components, of various mixer faucet fittings were an excellent substrate for the growth of legionellae. After replacement of all rubber washers with a National Water Council (U.K.)-approved material, legionellae could no longer be cultured.

Stagnation and obstruction to water flow in a VA hospital contributed to the buildup of legionellae in hot water storage tanks and to colonization of showerheads and faucets with legionellae (Ciesielski et al., 1984). When 30 aerated faucets were examined, 22 yielded L. pneumophila serogroup 1 whereas none of 26 unobstructed units was positive ($p<0.001$). Nine of 30 samples of water from aerated faucets, 11/30 from the pipe, and 17/30 from the aerator screen were positive. Nine of the aerators were sterilized and replaced but they were recolonized by L. pneumophila within 2 months.

Meenhorst et al. (1981) could find no obvious source for airborne spread of legionellae in Leiden University Hospital other than the water system. From swabs of showerheads, hot tapwater, and boiler water at a temperature of 54°C, they isolated a distinctive strain (Le-1) of L. pneumophila. In 6/8 patients this strain was either cultured or there was serologic evidence of infection caused by it. Strain Le-1 was isolated by direct plating from 10/11 hot water samples.

Stout et al. (1982) attempted to relate the presence and distribution of L. pneumophila serogroup 1 within the plumbing system of a hospital to the cases of LD that were occurring. They observed that L. pneumophila was present in potable water to which 5/6 patients with nosocomial LD were directly exposed. An ongoing surveillance of the hospital for LDB was initiated just before an outbreak in which 14 cultureconfirmed cases were diagnosed over a 3-week period. The surveillance extended over a period of 4 months during which 10 sites were tested monthly (Table III-7). LDB were isolated from 9/15 showers and 24/35 faucets. All sites were positive just before the outbreak and six of them remained positive during the entire

TABLE III-7

Presence or Absence of *L. pneumophila* in a Monthly Survey of 10 Water Sites in the Hospital[a]

Site[b]	Test Result			
	April[c]	May	June	July
SICU, faucet	+	+	+	+
SICU, faucet	+	+	+	+
MICU, faucet	+	+	+	+
4E, shower	+	+	+	+
7W, shower	+	-	-	-
8W, shower	+	-	-	-
9E, shower	+	-	-	-
9E, faucet	+	+	+	+
9W, faucet	+	+	+	+
11E, shower	+	-	-	-

[a] Source: Stout et al., 1982. Reprinted by permission of the New England Journal of Medicine, 306: 466-468.

[b] SICU denotes surgical intensive-care unit; MICU, medical intensive-care unit. E and W refer to the east and west wings of the hospital.

[c] Nosocomial outbreak of legionnaires' disease occurred in April.

4-month period. Forty-six of 51 isolates of L. pneumophila were serogroup 1; five were serogroup 5. The concentration of LDB ranged from 5-300 CFU per plate. In a previous study a comparison was made of the concentration of LDB in hot vs. cold water taps in this hospital (Stout et al., 1981). Nine positive samples from hot water sites gave a maximum plate count of 100 CFU per plate, whereas three positive samples from cold water sites gave a maximum of 2 CFU. Presumably, LDB entered the hospital water holding tanks from the reservoir. The bacteria then found a habitat suitable for growth in the storage tanks and the hot water system. Pressure changes dislodged the sediment, and a charge of potentially infectious organisms was carried to the faucets, showers, etc.

Cordes et al. (1981) examined showerheads in three different hospitals for contamination with LDB. In Chicago, where three patients had LD caused by L. pneumophila serogroup 6, 9 of 16, showerheads were positive for this organism. The three patients had showered 2-10 days preceding appearance of symptoms of LD. In Pittsburgh, L. pneumophila serogroup 1 was found; in Los Angeles, serogroups 1 and 4. In both places patients were being infected with the same serogroups. LDB were also isolated from showers in areas where no legionellosis was occurring. Sterilizing the showerheads with ethylene oxide did not prevent recontamination with LDB, suggesting that the potable water system was the source of the organisms.

In Melbourne, Australia, two nonfatal cases of LD caused by L. pneumophila were associated with the patient's exposure to showers in a reception ward during the incubation period of their disease. L. pneumophila was isolated from the shower water whose source was a holding tank maintained at 40°C (Makela et al., 1981).

Tobin et al. (1981b) surveyed the water systems of 28 hospitals and hotels in England and Wales, and three hotels abroad, for the presence of LDB. Forty-two isolates of L. pneumophila were obtained from cooling towers, showers, hot and cold bath water, faucets, and storage tanks in 31 sites, 6 of which were associated with cases of LD. Each of the water distribution systems at the six LDB-associated sites and the cooling towers at the three sites having them proved positive. In the other sites, water from 3/9 cooling towers, 4/24 taps or showers, and 1/15 storage tanks contained Legionella. Nevertheless, attempts to isolate legionellae from the main water supplies were unsuccessful (Tobin et al., 1981a, 1981b).

A well-supported argument for the key role of hot water tanks in maintaining LDB in potable water systems was made by Wadowsky et al. (1982). They cultured samples of sediment from plumbing fixtures in two gymnasiums, five private homes, and three local hospitals. Faucets and showers were also examined. L. pneumophila serogroups 1, 5, and 6 were recovered from plumbing fixtures of the hospitals, gymnasiums, and from one home. Their quantitative data on serial sampling of hot water tanks is presented in Table III-8. Quantitative data on sampling as related to temperature of the tank water is shown in Table III-9. Counts of L. pneumophila serogroups 1, 5 and 6 ranged from 7-13,850 CFU/mℓ. Legionella was isolated from tanks in which the water temperature was maintained in a range of 30-54°C, but not from those maintained at 71-77°C.

In the Pittsburgh VA Hospital where L. micdadei, the Pittsburgh pneumonia agent, was first implicated as a cause of legionellosis (Pasculle et al., 1979), this species was isolated from a hospital shower (Brown et al.,

TABLE III-8

Isolation of L. pneumophila from Hot Water Tanks[a]

Building	Number Samples	Temp (°C)		L. pneumophila CFU/mℓ		
		Tank[b]	Sample 1[c]	Sample 1	Sample 2[c]	Serogroup
Hospital A	2	54	31, 38	7; 1,340	0	1; 6
Hospital B	4	31-54	28-33	80-600	0-30	1; 6
Hospital C	6	43-45	ND	570-13,850	60-8,750	1; 5
	2	71; 77	ND	0	ND	NA
Gymnasium 1	2	43	34; 48	220; 310	0; 10	1; 5
Gymnasium 2	1	49	43	80[d]	0	1; 6

[a] Source: Wadowski et al., 1982

[b] Temperature at which hot water was maintained in tank for use.

[c] Sample 1 was collected immediately after drain valve at bottom of tank was opened; sample 2 was collected 2-3 minutes later.

[d] Sample also contained 20 CFU of L. micdadei per milliliter.

ND = Not done

NA = Not applicable

TABLE III-9

Isolation of L. pneumophila from Hot Water Tanks in Hospital C and Gymnasiums[a,b]

	Sample 1		Sample 2	
Hot Water Tank	Temp (°C)	CFU/mℓ	Temp (°C)	CFU/mℓ
Hospital C				
1	43	750	35	440
2	49	1,210	27	1,460
3	43	570	38	80
4	43	1,100	43	0
5	43	3,360	38	0
6	44	13,850	34	3,460
Gymnasiums				
1	48	220	30	850
2	34	310	30	300
3	43	80	38	270

[a] Source: Wadowski et al., 1982

[b] The interval between collection of samples 1 and 2 was 2 months for hospital C and 2.5 weeks for gymnasiums.

1982b) in a room where a patient developed pneumonia from L. micdadei. This organism was also isolated from a hot water tank in a gymnasium.

Bartlett et al. (1983) conducted a collaborative study of legionellae in hospital and hotel water supplies on behalf of the British Public Health Laboratory Service. Water systems in hotels, hospitals, and other large buildings in the United Kingdom were analyzed by direct culture. Sampling sites included cooling towers, humidifiers, storage tanks, hot water tanks, taps, and showers. A total of 1538 water samples were examined of which 314 (20%) were positive and 74 of these yielded more than one species of legionellae. In one of the collaborating laboratories, water from 36/54 hotels and hospitals contained legionellae, although only four institutions were associated with cases of legionellosis. The frequency of occurrence of the various species and serogroups encountered is shown in Table III-10.

A recent outbreak of 24 cases of LD pneumonia over a 10-month period occurred in immunosuppressed patients in an Iowa hospital (Helms et al., 1983). There were 11 fatalities (46%). Twenty-one patients were in a new hospital addition and 16 of these were in a new hematology-oncology unit. L. pneumophila serogroup 1 was isolated from 7/14 sink faucet swabs, 1/14 showerhead swabs, 6/9 sink water samples, and 1/2 hot water storage tanks, but not from the cold water supply. Water quality standards for free chlorine and bacterial plate counts were within acceptable limits for both hot and cold water during the sampling period.

L. pneumophila serogroup 1 was cultured from multiple samples of hot water, a hot water tank, and showers on two floors of a community hospital

TABLE III-10

Legionella in Water Systems in the United Kingdom (U.K.)*

Organism	Number of Positive Samples
L. pneumophila	503
Serogroup 1	156
Serogroup 2	21
Serogroup 3	49
Serogroup 4	85
Serogroup 5	30
Serogroup 6	38
Unknown	24
L. micdadei	1
L. bozemanii	19
L. gormanii	0
L. dumoffii	4
L. jordanis	2
L. longbeachae	1-22
L. wadsworthii	0
L. oakridgensis	0
Other (not yet identified)	7

*Source: Bartlett et al., 1983

experiencing six serologically confirmed cases of legionellosis (Hanrahan et al., 1984). There was a significant association between illness and length of hospitalization and between illness and distance from hospital room to the showers. Chlorination of the hot water system and cessation of shower use stopped the outbreak.

Hotels, Clubs, Public Buildings, Homes, and Factories -- The index hotel outbreak of all time was that at the Bellevue-Stratford Hotel in Philadelphia in 1976 that resulted in 182 cases of LD of which 29 were fatal (Fraser et al., 1977). The source of the infection was not discovered. Another outbreak (1974) at the same hotel preceded that in 1976 and was documented retrospectively by serology (Terranova et al., 1978).

An epidemic occurred at a hotel in Wisconsin where infection probably resulted from exposure to a Legionella-contaminated aerosol from a cooling tower. The exhaust from the tower was drawn down a chimney into a meeting room (Band et al., 1981).

A hotel in Benidorm, Spain, was the apparent site of exposure to fatal pneumonia for three tourists who visited there in 1973 (Reid et al., 1978) and of another tourist 4 years later (Lawson, 1978). Illness involving respiratory symptoms was reported by 86/164 (52%) of tourists staying at this hotel compared with 31/82 (38%) of those who stayed at other hotels in Benidorm. From 1973 to 1980, occasional cases of legionellosis continued to be associated with residing at this hotel (Meyer, 1983).

An epidemic of legionellosis in Bloomington, IN, was associated with the Indiana University Memorial Union building where visitors to the campus were housed (Politi et al., 1979). Legionellae were isolated from the cooling tower and also from environmental habitats.

While investigating four cases of pneumonia, three of whom had significant titers to L. pneumophila, it was found that all had stayed at the same English hotel (Tobin et al., 1981b). The four patients were members of a golfing party of 58 members. None of 26 golfers who stayed at other hotels was ill. Retrospectively, four other cases of pneumonia were discovered among persons who had stayed at this hotel. L. pneumophila serogroup 1 was demonstrated in the post mortem lung tissue of one of these cases. The same serogroup was isolated from water storage tanks, hot and cold taps, and shower outlets, but not from the water supply mains or from tapwater in five other buildings in the neighborhood. The water distribution system was disinfected and a continuous chlorination unit installed. No further cases have occurred.

De Lalla et al. (1980) reported five confirmed and two presumed acute cases of legionellosis (L. pneumophila) affecting persons visiting a particular hotel in Ravenna, Italy. One case was fatal. Water samples from several showers and sinks in guest rooms and from a surface pool were positive by DFA tests for L. pneumophila serogroups 1-4 (U.S. PHS, 1981).

When an outbreak of legionellosis (L. pneumophila serogroup 1) in St. Croix, Virgin Islands, was investigated, it was found that 12/21 cases had stayed at one hotel (Schlech et al., 1983). Cultures of serogroups 1 and 3 were isolated from several sites in the fresh water system of this hotel but also at three of four other hotels sampled. High seropositivity rates among residents at St. Croix suggested that the area may be hyperendemic for legionellae infection.

In Johannesburg, South Africa, a survey of cooling towers of air-conditioned hotels, cinemas, and office buildings; of hot water taps from a hospital; of underground water in a gold mine; and of storage tanks at a Zambian copper mine resulted in isolation of legionellae from all sources (Mauff and Koornhof, 1984).

The Pontiac, MI, outbreak in 1968 occurred in a county health department building as previously discussed (Glick et al., 1978). The source of infection was an aerosol produced by a contaminated evaporative condenser.

An outbreak of legionellosis affected 14 of ~1000 workers in a 6-month-old office building in San Francisco (Conwill et al., 1982). The illness was temporally associated with the operation of the building's air-conditioning system that was contaminated with L. pneumophila serogroups 1, 4, and 6. The air-conditioner was near an area of recent excavation. Only serogroup 1 was associated with the outbreak.

An outbreak of L. pneumophila in Atlanta, GA, involving golfers was epidemiologically related to the aerosol drift from a contaminated evaporative condenser located at the country club building (Cordes et al., 1980a).

An examination of tapwater and of scraping from a showerhead and faucet in each of 52 residences in Chicago revealed a high incidence of contamination by legionellae in the absence of clinical pneumonia among the occupants (Arnow and Weil, 1984). The residences were in 33 apartment buildings adjacent to a hospital with known contamination of its hot water system. L. pneumophila of five different serogroups was isolated from 19 (37%) of the residences but only from hot tapwater (≤55°C); no isolates were obtained from 15 systems at higher temperatures. Concentrations of legionellae ranged from 3-1000 CFU/ℓ. The hot water tanks were large institutional-type units dissimilar to the average domestic tank. Therefore, similar findings of legionellae may not apply to smaller domestic hot water tanks.

An interesting cluster of four confirmed and three presumptive cases of LD occurred in factory workers in Jamestown, NY (Shands, 1984). Cases appeared to be related to working in one particular building or in a second building across the street and downwind from the first. Both a cooling tower and a grinding machine located at the first building were positive for L. pneumophila serogroup 1, the same serogroup infecting the patients. The grinder used a water-oil mixture for cutting and during operation generated a sizable aerosol spray. It was not possible to prove whether the grinder or cooling tower or both were involved in disease transmission. Apparently this is the first outbreak of LD documented in a factory setting.

In Paris, France, 140 water samples consisting of 42 from air-conditioning equipment and 98 from plumbing fixtures in 18 buildings were cultured for LDB (Desplaces et al., 1984). None of the samples was associated with

LD outbreaks. Forty-five of 140 (32%) of the samples were positive; 27/45 contained two or three different legionellae. The breakdown of positive samples was as follows: 4/6 air-conditioning systems; 13/15 cooling towers; 1/27 airwash systems; 9/62 cold tapwater samples; 11/17 hot water samples; and 11/11 hot water tanks. Cold tapwater counts of legionellae were as high as 10^5 CFU/ℓ. L. pneumophila serogroup 1 was the most common serogroup isolated (28/58), although strains belonging to serogroups 2-6 of L. pneumophila as well as L. longbeachae serogroup 2, and three unclassified legionellae were isolated. Despite the gross contamination of the plumbing system, no isolates of legionellae were obtained from eight samples of mains water supplies. The above citations provide strong support to the theory that human exposure to potable water containing viable legionellae may cause legionellosis.

Cooling Towers. The first clue to the ecological habitat of the legionellae came during the investigation of the outbreak of Pontiac fever in 1968 (Glick et al., 1978). Epidemiologic analysis implicated an airborne agent and pointed to a defective evaporative condenser as the point source of the etiologic agent. In 1977 L. pneumophila was isolated from frozen tissues of guinea pigs exposed 9 years earlier to the air of the outbreak building and to aerosols of the contaminated condenser water.

The outbreak of LD in Bloomington, IN, in 1977-1978 suggested a cooling tower as one, but not the only, possible source of the organisms (Politi et al., 1979). Morris et al. (1979) examined 24 environmental samples of which seven were positive for Legionella. One water sample from the cooling tower, three water samples from a nearby stream, and three mud samples from the same stream yielded L. pneumophila of serogroups 1 and 3, and a culture later described as a new species, L. jordanis. This success indicated that the legionellae may be widely distributed in nature.

In 1978 a common source outbreak of LD at a Memphis hospital was clearly related epidemiologically to the use of an auxiliary air-conditioning cooling tower (Dondero et al., 1980). The outbreak started when the tower was put into operation and ceased when it was taken out of service. L. pneumophila was recovered from two samples of tower water. Airflow studies also showed that spray from the tower drifted into the areas having the highest attack rate.

Probably the largest epidemic of legionellosis that has occurred outside the United States took place in Vasteras, Sweden (Nordstrom et al., 1983). A total of 58 cases constituted the outbreak in late August and September 1979. Another 10 sporadic cases occurred in June, July, and early August preceding the epidemic. All patients (68) had stayed in Vasteras at some time in a 2-week period before becoming ill. The cases were documented serologically (IFA) and by isolation of L. pneumophila serogroup 1 from three patients. This bacterium also was recovered on numerous occasions from water taken from a cooling tower on the roof of an indoor shopping center visited by 57 of the cases during the presumed incubation period (14 days). According to Dr. S. Bernander, the epidemic appeared to be closely linked to the shopping center (Bernander, 1983). The air intake for the mall was adjacent to the cooling tower, and on hot days an aerosol was produced from the towers. Using a tracer, Bernander (1983) showed that the aerosol could be drawn into the air intake to the mall.

The entire question of sporadic legionellosis has gone begging for an answer insofar as community sources of infection are concerned. More attention should be paid to the many enclosed malls in the United States whose decor frequently includes a number of rippling waterfalls and bubbling, cascading fountains saturating the air with aerosols. These malls are visited by hundreds of thousands, perhaps millions, of people each week, and L. pneumophila has been isolated from mall pools (Cherry, 1983). The saving feature of these attractions may be that the temperature of the water is low enough to prevent the growth of critical inocula of legionellae.

An epidemic was reported by Cordes et al. (1980a) in which golfers apparently were infected with L. pneumophila emanating from an evaporative condenser on the club house. The tees and practice greens were rather close and downwind from the drift of the condenser. L. pneumophila serogroup 1 was isolated from the evaporative condenser, and a serogroup 3 culture was obtained from a moist soil sample on a creek bank of the golf course.

Band et al. (1981) described an epidemic of LD in which infection resulted from cooling tower exhaust (on the roof of a hotel) being drawn down an open chimney into a meeting room. The attack rate for those exposed to this meeting room was 1.5% but 0% for those exposed to other meeting rooms. L. pneumophila serogroup 1 was isolated from patients and from the cooling tower water and from a puddle of rainwater on the roof of the building.

A biphasic outbreak of legionellosis occurred in Burlington, VT, in 1980 (Klaucke et al., 1984). Disease was related temporally and epidemiologically to a contaminated cooling tower on a building 150 m upwind from the side of the hospital on which the highest rate of infection occurred. The phase one epidemic ceased when the contaminated tower was chlorinated and _Legionella_ could no longer be cultured from it. The second phase outbreak started when the tower again became positive. Following the second outbreak the tower was dismantled and replaced with a closed system. An interesting facet of this investigation was the effect on two maintenance workers who had direct contact with the contaminated cooling tower when it was accidentally turned on. One, standing by the fan, developed acute clinical LD whereas the other, not so heavily exposed, developed Pontiac fever. The distance over which airborne transmission occurred in these epidemics was greater (150 m or more) than that reported in other outbreaks, i.e., 15 m (Dondero et al., 1980) and 30-50 m (Cordes et al., 1980a).

Frequent contamination of cooling towers with legionellae is well documented (Bettinger et al., 1980). In a survey of 12 cooling towers and six chilled water systems in six states, 15/18 sources were positive by DFA tests and some samples were lethal to guinea pigs but no isolates of legionellae were obtained. Tyndall et al. (1983) isolated several strains of legionellae from large industrial cooling towers in Minnesota and Pennsylvania. These proved to be a new species, _L. oakridgensis_ (Orrison et al., 1983b).

Kurtz et al. (1982) examined 14 cooling water systems in London for legionellae and found 6 positive at the beginning of the study in April and

May and 10 positive at the end of the study in late summer. *L. pneumophila* serogroup 1 was the only serogroup or species isolated. Although treatment of the towers with various regimens of biocides was in progress during this period, legionellae were not eliminated or were eliminated only intermittently.

The most thorough study of legionellae in open recirculating cooling tower systems and evaporative cooling air-conditioners of both open- and closed-chilled water types was that of Fliermans et al. (1982). The key factor in maintaining a detectable level ($>9.1\times10^3/\ell$) of legionellae contamination was the nature of the source water; i.e., the more contaminated the source water, the higher the level of legionellae in these systems. Source waters included well water and public and private potable water. Densities of legionellae were determined by quantitative DFA techniques (Fliermans et al., 1981a) and were greatest in towers receiving surface water and least in those supplied from underground wells. Apropos of this, Spino et al. (1984) were unable to isolate legionellae from groundwater being "stripped" by aeration to remove volatile organic chemicals. All towers that were tested by Fliermans et al. (1982) were contaminated. In some towers the levels of *L. pneumophila* (serogroups 1, 2, 3 or 4) rose to 10^8-$10^9/\ell$ of cooling tower water. In one tower, 98% of the legionellae were *L. pneumophila* serogroup 1. Chlorination was used to maintain a residual of 1-1.5 mg/ℓ for over 200 hours with the objective of reducing the level of legionellae contamination to approximately that of the source water. When that level was achieved, a normal treatment of 0.7 mg/ℓ free residual chlorine for 1 hour on a daily basis maintained the density of legionellae at source water levels.

Air-conditioning units were shock-treated (50 mg/ℓ/day) with calcium hypochlorite and the effect on legionellae densities was determined. The legionellae were reduced to source water levels after 23 days of treatment. Fliermans et al. (1982) stated that elimination of legionellae from cooling towers and air-conditioning systems is an unreasonable objective when the source water is contaminated. The desirable goal is to maintain the systems so that legionellae densities approximate those naturally present in the water supply. This can be done by appropriate chlorination procedures.

The authors also noted that sentinel guinea pigs placed in an aerosol plume of a tower containing 10^8-10^9 *L. pneumophila*/ℓ by DFA tests developed legionellosis. Neither the number of animals nor the number of tests was given. Apparently, no isolations were made and no data were given on the effect on animals of the less heavily contaminated towers. More acceptable data would have included representative isolates of *L. pneumophila* obtained during the course of the study.

Saito et al. (1984) isolated 166 cultures of legionellae from 126/343 (36.7%) samples of cooling tower water obtained from sites widely distributed in Japan. *L. pneumophila* was the most common species (127 isolates) and serogroup 1 the most common serogroup (110 isolates). Twelve cultures of *L. bozemanii* were obtained and the remainder (27) were unidentified *Legionella*-like organisms.

Nakahama (1983) isolated 16 strains (66.7%) of *L. pneumophila* from 24 water samples collected from cooling towers in the Okayama prefecture of Japan.

The above examples illustrate the role of cooling towers contaminated with legionellae in production of disease. In several other epidemics and in sporadic cases, circumstantial evidence indicated a connection between contaminated cooling towers and legionellosis but epidemiologic data were lacking.

Nebulizers, Humidifiers and Whirlpools. The first report of contamination of couplant reservoirs of ultrasonic nebulizers with legionellae was that of Gorman et al. (1980). Tapwater from the reservoirs of seven nebulizers used in three hospitals was examined by culture, guinea pig and chick embryo inoculation. L. micdadei was isolated for three nebulizers, each from a different hospital. The importance of this discovery was as follows: 1) The contaminated nebulizers were not proved to be a source of infection; 2) the rather uncommon PPA, like other legionellae, existed in aquatic habitats; and 3) L. micdadei was isolated from a useful piece of hospital equipment that, when not operated properly, harbored other organisms that may cause nosocomial pneumonias.

Over a 2-year period, 36 cases of nosocomial LD occurred in patients in a Connecticut hospital (Jones et al. 1984). Illness could not be environmentally related except to prior respiratory therapy including aerosol masks, nebulizers, or positive-pressure breathing equipment. Four different serogroups of L. pneumophila were demonstrated in the patients and in some of the respiratory therapy equipment. Two of these serogroups were found in many samples of the potable water that was used to rinse the equipment.

Water from a hospital humidifer was found to be contaminated with L. pneumophila serogroup 1 (Zuravleff et al., 1983). Sixteen culture plates were placed in a closed room with the humidifier turned on for 16 hours. All 16 plates were positive for serogroup 1. Sentinel guinea pigs exposed to the humidifier for 3-10 days seroconverted (44%), but pneumonia did not develop and legionellae were not isolated. Transmission of LDB by humidifier aerosols may cause nosocomial legionellosis. Legionellae had previously been found to be ubiquitous in the water supply of this hospital when it was experiencing endemic LD (Stout et al., 1982).

The water and sediment from a room humidifier in a police communications center in Ohio were highly contaminated with L. pneumophila (Cherry, 1980). At least one case of pneumonia and, perhaps, other cases of respiratory disease may have occurred among persons occupying the room and may have been caused by aerosols from the humidifier. The concentration of Legionella in the sediment of the humidifier was 10^8/mℓ and remained at a high level throughout several months of wet storage at room temperature.

Arnow et al. (1982) investigated five cases of nosocomial LD in which the patients had received treatment with aerosolized tapwater during a 2- to 10-day incubation period preceding illness. Four patients used a jet nebulizer; one patient, a portable room humidifier. L. pneumophila (serogroups 1 and 6) were isolated from the tapwater and from respiratory devices filled with tapwater, as well as from the patients. More isolates (10) were obtained from hot water than from cold (1). Free chlorine levels were 0.3-0.4 mg/ℓ for cold tapwater and 0.05 mg/ℓ for hot tapwater.

Contamination of a whirlpool by L. pneumophila serogroup 6 induced an acute self-limited illness (Pontiac fever) in 14 of a group of 23 females using the pool, but not in other women of the same group who did not use the pool (Remis et al., 1983). Patients spent significantly ($p=0.04$) more time in the pool than did the controls. Eleven of 14 cases seroconverted primarily to serogroup 6, whereas only 3/14 controls serocoverted. Whirlpools may be a reservoir for legionellae and L. pneumophila serogroup 6 may cause Pontiac fever.

Investigation of a possible outbreak of legionellosis at an inn in Vermont revealed that recreational whirlpool spas at this and two other inns nearby were contaminated with L. pneumophila serogroups 1 or 6 or both (Witherell et al., 1983). Water samples from the swimming pool, water system, showerheads and dehumidifiers were negative. The source of contamination for the whirlpools was not found.

Summary

Legionella and legionellosis are an international problem because the bacteria and the disease do not appear to have geographic, climatic or ethnic limitations. The organism and the disease have been reported most frequently from the developed western countries reflecting greater experience of medical personnel in recognizing the disease and greater sophistication of techniques used by laboratory workers for isolation and identification of the etiologic agent. Very few cases of legionellosis have been reported from tropical countries, but the Puerto Rican experience shows that legionellae are present and that legionellosis occurs. Because the legionellae appear to thrive in warm aquatic environments, one suspects that tropical surface waters are an excellent habitat for them. Although legionellae have been isolated from estuarine waters in Puerto Rico, there is, as yet, no evidence that the marine environment is their normal habitat. Their sensitivity to sodium ions may, in part, explain this.

In the United States, legionellosis is a reportable disease. The number of cases reported in 1978 and 1979 were the highest until 1982 and 1983. Reports for the first 2 years were undoubtedly inflated by the widespread search for retrospective cases based on examination of preserved lung tissue from pathology libraries. The largest number of cases were those reported in 1982 and 1983, perhaps reflecting the increasing awareness of medical personnel and the development and greater availability of more suitable isolation media and reagents. From the available data, which include both epidemic and sporadic cases, the number of reported cases for the 6-month periods of May through October is more than twice that for the other 6 months of the year--the winter months during which other respiratory diseases are most prevalent and severe. If the Seattle experience is applicable, an estimate of 25,000 sporadic cases of legionellosis per year is not unreasonable.

The frequent occurrence of legionellae in surface waters has evoked little interest insofar as infection is concerned, although logic dictates, and experimental data suggest, that these waters are the ultimate source of infection. The aerosols of cooling towers and evaporative condensers utilizing either raw or potable waters are proven sources of legionellosis outbreaks, especially when accessed by a susceptible population of immunocompromised persons or by normal individuals positioned to receive overwhelming exposure.

Of great concern is the proven contamination by legionellae of water distribution systems such as those of hospitals. The data suggest that these organisms gain entrance to plumbing equipment from community water distribution systems. They may survive water plant treatment procedures including chlorination, allowing them to reach distribution points where a decreased or negative chlorine residual permits concentration and amplification.

Concentration in cul-de-sacs, in bypass piping, in intermittently used components, and, especially, in hot water tanks with subsequent deposition on faucet and shower sieves, washers, valves, etc., creates a hazardous situation for susceptible hosts. Legionellae are aerosolized from these colonized surfaces during normal activities such as showering and drawing water from faucets, especially those equipped with aerators.

Humidifiers, respiratory therapy equipment, and whirlpools also are potential sources of Legionella infection. Humidifiers accumulate slime and sludge that act as a substrate for bacterial growth. Respiratory therapy equipment requires frequent decontamination. Tapwater should not be used in an ultrasonic nebulizer chamber or even in the couplant chamber. Whirlpool spas have been associated with outbreaks of legionellosis in which the water and filters of the whirlpool have been found contaminated with legionellae. In one outbreak good epidemiologic data indicated that the patients were, indeed, infected from a whirlpool spa, although the disease resembled Pontiac fever rather than legionellosis.

The role of algae in supporting growth and viability of legionellae in aquatic habitats has not been well defined although they appear to exert a significant stimulus. Algae inhabit cooling towers and may cause a serious fouling problem if the equipment is not well maintained. There is convincing evidence that several species of amoebae serve as hosts for legionellae often with considerable host specificity. Legionellae invade the amoebae and multiply within the animal's vacuoles much as they do in the macrophages of higher animals. When the amoebae die, legionellae are liberated.

The role of soil in transmission of Legionella infection is still open to question. Although excavations or other soil disturbances were associated with some epidemics, there are no convincing data that soil was involved in the transmission of infection. Furthermore, legionellae have been isolated only from mud or moist soil and these are rare occurrences.

Before selective media were available for the isolation of legionellae from environmental samples, legionellae-like organisms were frequently observed by DFA staining in soils and, especially, in earthworms and some soil arthropods. Because legionellae were not isolated, it is not known whether they also have a terrestrial habitat or if antigenically related nonlegionellae bacteria were being observed. If we are to understand the ecology of legionellosis, these studies should be repeated using more efficient modern procedures.

IV. METHODOLOGY

Collection from Water

Two basic methods with several variations have been used to collect legionellae from water samples. The method used depends upon the putative legionellae content of the sample, the source of the sample, its physical nature, and the level of sensitivity desired. The methods used most are either concentration by centrifugation or filtration.

Morris et al. (1979) obtained total plate counts on samples of cooling tower water, air-conditioner condensate, and surface water and soil before injecting the samples into guinea pigs. The total plate count was used as a guide to the relative microbiologic contamination of the sample and, therefore, to the size of the inoculum that could be tolerated by guinea pigs. When the contamination was minor, the samples were concentrated by centrifugation and the sediment was injected into guinea pigs. If the samples were highly contaminated, guinea pigs were injected with appropriate dilutions of the samples.

Fliermans et al. (1981a) processed a large number of surface water samples of 20 ℓ volumes by continuous flow centrifugation. The sediment was concentrated (500-fold) in 40 mℓ of water, ~3 mℓ of which was injected i.p. into guinea pigs if smears of the sediment examined by DFA staining tests indicated the presence of legionellae. The DFA procedure was standardized so that the observation of a single typically stained cell indicated an LDB concentration of $9.1\times10^{3}/\ell$ of legionellae. Appropriate volumes of samples that were strongly positive were then injected i.p. into guinea pigs for attempted isolation of the legionellae. These concentrated samples were not plated directly on media because the total microflora overloaded the media. This standardized DFA procedure was also used for quantitative estimates of the densities of legionellae in cooling towers (Fliermans et al., 1982; Tyndall et al., 1983).

Legionellae were isolated from ~15% of the DFA-positive water samples (Fliermans et al., 1981a). This could have been caused by the presence of nonviable cells, by serologically related non-Legionella, by lack of virulence for guinea pigs, or by inhibition and overgrowth of legionellae by the diverse microbiological flora in the water samples. The question of viability of DFA-positive cells was explored by using the dye INT (1-p-iodophenyl-2-p-nitro-phenyl tetrazolium chloride). If viable cells were present, the INT dye would be transported intracellularly where it would be precipitated as dark-red aggregates. Simultaneous examination of DFA and INT stained cells identified them both as legionellae and as viable legionellae (Fliermans et al., 1981b). Thus, in situ determination of viability allows an assessment of the activity of legionellae in their natural habitat and at different temperatures, pH levels, etc.

Detection of legionellae by DFA staining of environmental samples containing a diverse bacteria flora is a valuable presumptive test for these organisms. The results, however, are subject to misinterpretation if legionellae cannot be isolated. Bacteria that are serologically related and morphologically similar to legionellae may give false-positive results (Orrison et al., 1983a). This apparently happens infrequently with L. pneumophila but there are, as yet, no data on possible serologic relationships of the other 21 species Legionella to non-Legionella bacteria. This situation mandates an awareness of the possibility of false-positive tests.

With the advent of improved isolation media, especially selective media, the processing of cooling tower waters was greatly simplified. Isolation of legionellae from surface waters continues to be difficult because, with rare exceptions, the ratio of nonlegionellae to legionellae is very high. Most samples from heat-rejection equipment now can be processed by filtration of 1-10 ℓ of water using pressure or vacuum filtration through membrane filters of appropriate diameters and porosities (Orrison et al., 1981; Tobin et al., 1981b; Swann et al., 1982; Christensen et al., 1984).

Detection of legionellae contamination of potable water and plumbing fixtures is a major concern of hospitals experiencing cases of LD. Concentration of the bacteria by filtration or centrifugation may be required, but the task is usually simplified by the clarity of the sample and low ratios of nonlegionellae and legionellae. Faucets, showerheads, sieves, filters, etc., are usually sampled by disassembling, swabbing, and scraping to obtain legionellae-bearing debris or scale (Helms et al., 1983; Edelstein et al., 1982b; Fisher-Hoch et al., 1982; Arnow and Weil, 1984; Fisher-Hoch et al., 1984; Klaucke et al., 1984). Water storage tanks and hot water tanks may be sampled most efficiently by collecting sediment and scale from appropriate areas. The water in the reservoirs of nebulizers should also be tested.

All of the above samples may either be plated directly on appropriate media or concentrated by filtration or centrifugation followed by plating or by injection into guinea pigs. The greatest efficiency is obtained by using all three procedures.

Some investigators have found that heat or an acid wash treatment enhances recovery of legionellae from environmental specimens (Edelstein et al., 1982b,c; Yee and Wadowsky, 1982; Groothuis and Veenendaal, 1983; Greaves, 1980; Bopp et al., 1981). Heating at 60°C for 1-2 minutes did not affect the survival of a majority of 13 strains of legionellae, but 60°C for 2 minutes reduced the numbers of *Pseudomonas aeruginosa* contaminants by 98% (Edelstein et al., 1982c). Heat treatment should not be used routinely because heating of contaminated clinical specimens at 60°C for 1-2 minutes before plating them was no better than plating them directly on selective media without heating. Legionellae are heterogeneous in their heat resistance. Also, legionellae in clinical samples do not appear to differ in heat resistance from legionellae isolated from hot water habitats (Edelstein et al., 1982c). Legionellae have been isolated from reservoir water and a swab from a cooling tower by inoculating the concentrated samples into tapwater and incubating them at 42°C for 1 week (Yee and Wadowsky, 1982). If pseudomonads or other contaminants were present, exposure of the samples at 45°C for 24 hours or at 50°C for 30 minutes may be beneficial for environmental samples as well as for clinical specimens. Groothuis and Veenendaal (1983) found that heating of concentrated environmental samples at 50°C for 30 minutes reduced the contaminating flora by a factor of $10^{1.6}$ to $10^{3.9}$, whereas legionellae were reduced only by a factor of 1.05-1.44. They recommended the routine use of this heat treatment for isolation of legionellae from both environmental and clinical specimens.

Dennis et al. (1984) compared heat treatment (50°C) and acid treatment of environmental water samples followed by plating on three different media for effectiveness in isolating legionellae. The media used were nonselective buffered charcoal-yeast extract (BCYE), and selective media consisting of BCYE supplemented with cefamandole, polymyxin B, and cycloheximide (BCYE CCP), and BCYE containing glycine, vancomycin, polymyxin B, and cycloheximide (BCYE GVPC). The latter medium was the most effective plating medium. Both pretreatment methods were effective, but heat treatment was recommended because of its simplicity. The investigators found that pretreatment of clinical specimens by heat was not effective.

Greaves (1980) was the first to report the use of an acid wash treatment to facilitate culturing legionellae but he did not evaluate the procedure. Wang et al. (1979) had noted earlier that L. pneumophila serogroup 1 was resistant to HCl at a pH of 2.0 for 30 minutes but lost viability fairly rapidly below pH 2.0. A detailed procedure for isolation of Legionella spp. from environmental water samples by acid treatment followed by plating on a selective medium was described by Bopp et al. (1981). They pretreated water samples, either concentrated by centrifugation or not concentrated, with an HCl-KCl buffer mixture at pH 2.2 for periods of 5-60 minutes. The samples then were plated on both nonselective CYE agar supplemented with L-cysteine and ferric pyrophosphate or on a selective medium (CCVC) consisting of CYE agar containing cephalothin, colistin, vancomycin, and cycloheximide. A third medium (CCV) was CYE agar supplemented with only the first three antimicrobials. The most isolations were obtained by acid treatment of centrifuged samples for 5 minutes followed by plating on either CYE or CCVC agars.

Direct plating or plating of unconcentrated samples on the same two media were less effective. The selective medium of Edelstein and Finegold (1979) was less effective than the CCV medium in allowing isolations of legionellae and in inhibiting competing flora in environmental water samples. The Edelstein medium consisted of CYE agar plus polymyxin B and vancomycin and was effective for isolation of _L. pneumophila_ from clinical specimens. The caveat to be observed is that a sample treatment and cultural procedure that is satisfactory for clinical specimens may not be suitable for environmental samples and vice versa.

A comparison of direct plating with guinea pig inoculation for isolation of _L. pneumophila_ from hospital potable water specimens showed that the sensitivity of the former was significantly ($p<0.01$) greater than animal inoculation (Edelstein et al., 1982b). Water samples were plated directly but, if results were negative, they were replated after concentration.

Culture Media

The basic medium used by most investigators for culturing legionellae from clinical or environmental sources is the CYE medium devised by Feeley et al. (1979). This is a CYE medium supplemented with L-cysteine and soluble ferric pyrophosphate, additives required for growth by most _Legionella_ spp. The medium has been improved by the addition of a buffer (N-2-acetamido-2-aminoethane-sulfonic acid) known as ACES (Pasculle et al., 1980). Buffering is important because the pH optimum for growth of legionellae in culture is very narrow. The BCYE medium was further improved by the addition of α-ketoglutarate (BCYEα), a Krebs cycle intermediate that is readily catabolized by these bacteria (Edelstein, 1981; Pine et al., 1979).

Legionellae may also be cultured in a variety of liquid media both synthetic and semisynthetic. A variety of formulae have been devised for Legionella media for special purposes. Several chemically defined media have been developed (Pine et al., 1979; Warren and Miller, 1979; Ristroph et al., 1981). A recent comparison of growth of one strain of L. pneumophila in 10 liquid media showed that the gonococcal-ferric-cysteine broth of Warren and Miller (1979) modified by the addition of 1% yeast extract and the omission of NaCl gave the best growth (Saito et al., 1981). Nine of 10 strains of L. pneumophila tested in this media gave excellent growth. Shaken cultures gave a faster growth rate and better yields (1 $\log_{10}$) than static cultures with 2.5% CO_2. No data were given on cultures shaken in an atmosphere of 2.5% CO_2.

Recently, a simple transparent medium containing yeast extract, monosodium glutamate, phosphate, L-cysteine, ferric pyrophosphate, and hemin was described (Johnson et al., 1982). A solid medium was prepared by the addition of agar and soluble starch. These media gave plating efficiencies comparable to those of CYE (apparently unbuffered) for the growth of 23 Legionella strains. These transparent media enhance the observation of colony morphology, are suitable for production of antigens, and facilitate the determination of antimicrobial susceptibilities.

For the study of algal-legionellae relationships, synthetic inorganic mineral salts media have been used in conjunction with algae or algal products (Tison et al., 1980; Pope et al., 1982; Bohach and Snyder, 1983a,b). A simple transparent medium suitable for antimicrobial assays, genetic studies, or production of biological reagents was described by Johnson et al. (1982).

Edelstein (1982) used BCYEα medium, BCYE with antimicrobial agents (BMPAα medium), and BCYEα with antimicrobial agents, glycine, and differential dyes (MWY medium) to culture 100 water samples, 95% of which were collected from hospital potable water fixtures. Samples plated on BCYEα and BMPAα media also were subjected to an acid wash procedure. The MWY medium detected 92% of all positive cultures followed by BCYE medium with acid wash (83%). The other combinations for pretreatment and media were less successful. The MWY medium was a modification of that described by Wadowsky and Yee (1981) and contained 0.3% glycine, 50 U of polymyxin B, 1 μg of vancomycin, 80 μg of anisomycin, 10 μg of bromthymol blue, and 10 μg of bromcresol purple per milliliter. Edelstein (1982) concluded that the MWY medium was the best of the three media tested for isolating *L. pneumophila* from potable water samples.

Keathley and Winn (1984) compared seven media, both commercial and laboratory prepared, for their ability to support the growth of the largest number of colonies in the shortest time when they were inoculated with clinical specimens. They found that BCYE with α-ketoglutarate (BCYEα) gave by far the most colonies in the shortest time. Two media, transparent yeast-extract-phosphate hemin agar and Greaves horse blood agar, neither containing charcoal, were completely unsatisfactory.

The formulae and details of preparation of the basic media as well as the procedures for recovery of legionellae from water are given in two laboratory manuals accompanying this criteria document. The manuals were furnished by Dr. James C. Feeley and by Dr. Paul H. Edelstein and are cited as follows: Gorman et al. (1983); Edelstein (1984). The Edelstein manual also contains a section on identification of Legionella species. The Gorman manual contains flow diagrams for examination of water by culture and by animal inoculation.

Serologic Tests

Serologic tests are of two kinds: tests for antigens and tests for antibodies. The former is applicable to both environmental and clinical specimens, whereas the latter is useful only for serologic diagnosis of disease in animals. The IFA test was first applied by McDade et al. (1977) to demonstrate the bacterial etiologic agent of LD. The DFA test was first used to detect *L. pneumophila* in smears and sections of lungs from fatal cases of LD (Cherry et al., 1978). Both tests are extensively used in the diagnosis of LD.

A variety of other serologic tests have been devised for detection of *Legionella* antigens or antibodies. These are listed in Table IV-1. They differ primarily in sensitivity, specificity, and predictive value. Some are complex, requiring many separate steps, i.e., the kinetic enzyme-linked immunosorbent assay (K-ELISA) or special reagents, i.e., radioimmunoassay (RIA). Some are quite simple, i.e., DFA or the slide agglutination test (SAT). The crossed immunoelectrophoresis (CIE) procedure is a tool that facilitates detailed quantitative and qualitative analysis of complex cellular antigens and their antibodies and its use is not likely to become routine.

TABLE IV-1

Serologic Tests for Legionella Antigens or Antibodies*

Serologic Test	Specimen	Substance Detected	Status of Test	References
DFA	Clinical-tissue, etc.; environmental-water, etc.	LDB cells or antigen	Routine	Cherry et al., 1978; Cherry and McKinney, 1979; Thomason et al., 1979b; Saravolatz et al., 1981
IFA	Serum	Antibody (IgG, IgM, IgA)	Routine (reference method)	Wilkinson et al., 1979a,b, 1981a
ELISA	Serum	Antibody (IgG, IgM, IgA)	Routine	Farshy et al., 1978; Wreghitt et al., 1982
ELISA	Urine	Soluble antigen	Experimental	Sathapatayavongs et al., 1982; Kohler and Sathapatayavongs, 1983
SAT	Culture	Species or serogroup identity	Routine (agrees with DFA)	Thacker et al., 1983
MAT	Serum	Antibody (IgM, primarily)	Relatively insensitive	Farshy et al., 1979; Harrison and Taylor, 1982; Bornstein and Fleurette, 1983

TABLE IV-1 (cont.)

Serologic Test	Specimen	Substance Detected	Status of Test	References
IHA	Serum	Antibody	97.3% agreement with IFA, 94.9% agreement with IFA	Yonke et al., 1981 Edson et al., 1979
LAT	Urine	Soluble antigen	Experimental (less sensitive than ELISA or RIA)	Sathapatayavongs et al., 1983
K-ELISA	Serum	Antibody (IgG)	Experimental (quantitative)	Sampson et al., 1983
RIA	Urine	Soluble antigen; early detection	Experimental	Kohler et al., 1981; Kohler and Sathapatayavongs, 1983
CIE	Soluble culture; antigens, serum, urine	Various cellular antigens or antibodies	Experimental	Joly and Kenny, 1982; Collins et al., 1983

*Source: Blackmon, 1981

DFA = Direct Fluorescent Antibody; IHA = Indirect Hemagglutination; IFA = Indirect Fluorescent Antibody; LAT = Latex Agglutination Test; ELISA = Enzyme-Linked Immunosorbent Assay; K-ELISA = Kinetic-Dependent ELISA; RIA = Radioimmunoassay; SAT = Slide Agglutination Test; CIE = Crossed Immunoelectrophoresis; MAT = Microagglutination Test

Monoclonal antibodies for L. pneumophila have been developed recently and hold great promise for the typing of isolated cultures and for identifying a common source of infection in epidemics and in nosocomial infections (McKinney et al., 1983). These highly specific antibodies are also useful for definitive typing of L. pneumophila in tissue sections from clinical cases of disease when cultural isolates are unavailable. They can be adapted to the detection of Legionella antigens in urine by the ELISA or RIA tests (McKinney et al., 1983).

Plouffe et al. (1983) used plasmid content and monoclonal antibody patterns to define three subgroups of L. pneumophila serogroup 1 cultures. Two subgroups were unique to cultures from hot water systems and patients in one hospital building in which the nosocomial case rate was 1.03/1000 discharges (19 cases). The third subgroup was unique to the second building with a case rate of 0.08/1000 discharges (1 case). This difference suggested that L. pneumophila from the first building was more virulent than the other. No differences were found in the water systems of the two buildings or in the numbers of L. pneumophila colonizing their hot water systems.

Meyer and Edelstein (1983) compared the sensitivity and specificity of the IFA, DFA, and cultural tests for the diagnosis of LD (Table IV-2). It should be noted that the specificity of the IFA test may vary outside the 90-95% range depending upon the species or serogroup of Legionella for which antibody is being measured and upon the demographics of the population whose sera is being tested.

TABLE IV-2

Diagnosis of Legionnaires' Disease[a]

	Sensitivity (percent)	Specificity (percent)	Comments	References
Seroconversion (IFA)	75	90-95	Acute and convalescent specimens needed up to 6-8 weeks apart	Edelstein, 1982[b]
DFA Sputum or TTA	50-75	99	Test serogroup specific[c]	Edelstein, 1982[b]
Lung	80-90	99		Edelstein, 1982[b]
Cultures[d]				
TTA	90-100	100	Special medium needed	Edelstein, 1982[b]
Blood	10-20	100	Special medium needed	Edelstein, 1982[b]
Lung biopsy	90-100	100	Special medium needed	Edelstein, 1982[b]
Sputum	80-90	100	Selective medium needed	Edelstein et al., 1982[b]
Urine antigen[e]	80-90	99	Test serogroup specific	Sathapatayavongs et al., 1982

[a] Source: Meyer and Edelstein, 1983. Reprinted by permission of Raven Press, New York.

[b] Based in part on incomplete and unpublished data.

[c] There is no cross-reaction among the 14 published antigenic types. If the wrong antibody is used, the sensitivity is 0%.

[d] The sensitivity of culture diagnosis has not been well studied because of the difficulty of case definition. On the basis of relative yield, culture diagnosis appears to be more sensitive than other methods of diagnosis.

[e] Radioimmunoassay or enzyme-linked immunosorbent assay.

IFA = Indirect fluorescent antibody testing; DFA = direct immunofluorescent antibody examination; TTA = transtracheal aspirate.

Summary

The examination of water for legionellae usually requires concentration of the bacteria by either centrifugation or filtration. Large volumes of turbid water are processed by continuous flow centrifugation; clear water may be filtered through large-diameter membrane filters. Most potable water samples are easily filtered or centrifuged and, frequently, they can be plated directly. Sediment, scale, and sludge from hot water tanks, holding tanks, faucets, showerheads, etc., often can be cultured directly or injected into guinea pigs for isolation of legionellae. When the ratio of nonlegionellae to legionellae is high, pretreatment of the sample by an acid wash or heat greatly enhances recovery of legionellae on culture media.

The basic culture medium for both environmental and clinical specimens is CYE agar or a modification thereof. The three major modifications have been 1) addition of ACES buffer, 2) addition of α-ketoglutarate, and 3) addition of various combinations of a number of antimicrobial agents. The buffer maintains the pH within the range that is critical for legionellae; the α-ketoglutarate stimulates growth; and the antimicrobials inhibit or prevent overgrowth of contaminants.

Recent experience indicates that culturing of specimens on properly prepared and standardized culture media is much more sensitive than guinea pig inoculation for isolation of legionellae. The difference is even more striking when one considers the much larger inoculum given the animal. Direct plating also allows isolation of a virulent legionellae; conversely, isolation by direct plating gives no information about the virulence of environmental cultures.

Surface waters, soils, and other highly contaminated samples may be processed more efficiently through guinea pigs and embryonated eggs because acid washes and heat treatment may insufficiently decontaminate the samples. If time and expense are not factors of importance, all of the techniques, including the use of chick embryos, may be used to maximize the chances for isolation of the legionellae.

The basic serologic tests for legionellae are the IFA test for serum antibody and the DFA test for LDB cells or antigen in tissue. Many additional serologic tests for both antigens and antibodies have been devised. They differ in sensitivity, specificity, and in predictive value. Some of the most useful are tests for Legionella antigen in urine and serum and some of the simplified agglutination tests for species and serogroup identification of legionellae.

Choice of the most appropriate methods for surveillance and control depends upon the type of specimen or sample being examined, the timeframe in which the results are needed, and the degree of specificity or sensitivity required. Most clinical specimens can be examined rapidly and with a high degree of specificity and sensitivity using DFA tests. Exceptions may be fecal specimens and, perhaps, oropharyngeal specimens. The availability of DFA reagents complementary to the legionellae of interest is, of course, a critical requirement. If suitable selective media are available, DFA testing appears less sensitive than culturing for the examination of most clinical specimens and potable water. Examination of urine or serum samples for soluble antigens of legionellae is a promising diagnostic approach.

For surveillance, potable water samples may be rapidly screened by DFA tests. Positive samples may be cultured to confirm the DFA results. Surface water should be screened by DFA tests and positive samples injected into guinea pigs for isolation of legionellae. Selective media may be appropriate in some cases, but overgrowth of culture plates by associated biota is a major problem. Culture methods using selective media have complete specificity if the isolates are correctly identified; sensitivity varies widely, being high with most clinical specimens, intermediate with water samples from sources like cooling towers, and of limited use with surface water samples.

V. INDICATORS FOR LEGIONELLA IN WATER

Fliermans (1983) stated that the search for indicator organisms for legionellae had been unsuccessful. In studies of the ecology of legionellae, an organism or some other indicator is needed that is easy to isolate, easy to enumerate, or easy to measure, and that will serve as a qualitative and quantitative clue to the presence of legionellae in water much as *Escherichia coli* does for fecal pollution.

In a survey of *Legionella* in both raw and treated water supplies, Tison and Seidler (1983) found the concentration of legionellae as measured by DFA staining to exceed both the standard plate count and the total coliform count by 2-3 log 10. These data are questionable because the authors were unable to isolate any legionellae; they attributed this failure to the low temperature (<13.5°C) of their samples.

Although legionellae have been isolated frequently from the sediment that deposits in the bottom of hot water tanks, there appears to be no correlation between the turbidity of the water-containing sediment and the presence or numbers of legionellae (Yee, 1984). There was no significant relationship between turbidity as measured by Secchi discs and the numbers of legionellae enumerated by DFA staining in 143 samples of surface water examined by Fliermans et al. (1981a).

Yee and Wadowsky (1982) found that naturally occurring *L. pneumophila* in tap water multiplied most rapidly at 42°C. Later, Wadowsky and Yee (1983) isolated *Flavobacterium breve* from a hot water tank where it was associated with *L. pneumophila* and showed that the organism supported satellite growth of *L. pneumophila* and three other species of legionellae on unbuffered CYE medium supplemented with ferric pyrophosphate but not with L-cysteine. They concluded that the *Flavobacterium* spp. and, perhaps, other nonlegionellae were supplying an essential nutrient (possibly L-cysteine) for the growth of the legionellae.

Subsurface satellite colonies of LDB were observed around colonies of *Hemophilus influenzae*, *Staphylococcus aureus*, and *Klebsiella pneumoniae* by Stout et al. (1983a). This was interpreted to indicate nutritional symbiosis. Stout et al. (1983b) found that a combination of sediment consisting of scale and organic particles from hot water systems, combined with an associated pseudomonad, was synergistic for growth of LDB. The pseudomonad apparently used the sediment for its nutrition and, in turn, stimulated LDB growth by providing L-cysteine.

The role of blue-green and green algae in stimulating growth and in enhancing the stability of legionellae was discussed in an earlier section of this document. Recently, Bohach and Snyder (1983b) reported that *L. pneumophila* adhered to the slime layer of a cyanobacterium (*Fischerella* spp.) and probably obtained nutrients from it, although the attachment was not a specific one. Algae are frequently found in heat-rejection devices together with legionellae, but they cannot be considered an indicator organism on the basis of present knowledge. Legionellae in hot water systems may be influenced by soluble organic nutrients produced by algae growing in reservoirs and transported through the distribution system, but substantial evidence for this is lacking.

The relationship between amoebae and legionellae is an intimate one as was discussed earlier in this report. It appears that some amoebae may be infected by some legionellae leading to multiplication of the legionellae, lysis of the amoebae, and release of the legionellae. This relationship certainly may exist in surface waters and cooling towers, but no data have been found indicating that it takes place in potable water distribution systems.

While studying legionellae in cooling towers in London, Kurtz et al. (1982) found a significant ($p<0.01$) relationship between high levels of total dissolved solids and chlorides and the presence of *L. pneumophila*. No correlation of legionellae with the presence of algae was observed. Clear evidence of an indicator for legionellae was not obtained.

Ortiz-Roque and Hazen (1983) reported a significant positive correlation between sulfate and phosphate concentrations and pH of surface and estuarine waters in Puerto Rico and the density of *L. pneumophila*.

In the lakes of the Mt. St. Helen's blast zone, the number of legionellae and the number of oxidizing and denitrifying bacteria in the vertical profiles of the lakes were similar (Tison et al., 1982). There was no correlation, however, between numbers of legionellae and total bacterial counts.

A thorough search for *Legionella* in water distribution systems in Columbus, OH, failed to demonstrate the bacteria in mainline service water although they were readily isolated from water samples of hot water recirculating systems and from cold water trunklines within large buildings (Voss et al., 1984).

Summary

Designation of any biological or chemical indicator for legionellae in water cannot, at this time, be supported by acceptable evidence.

VI. HEALTH EFFECTS IN ANIMALS

The susceptibility of the lower animals to infection by legionellae was discussed in the Host Range Section in Chapter II. Guinea pigs, rhesus monkeys, marmosets and rats are the only Legionella hosts for which sufficient information exists to warrant discussion. Guinea pigs have been studied extensively.

Acute Disease

Guinea Pigs. Baskerville (1984) compared experimental Legionella aerosol infection of guinea pigs with naturally occurring human disease that was presumably also induced by aerosols. The similarities of the disease in the two species are shown in Table VI-1. The mortality rate for untreated legionellosis in humans is generally in the 10-20% range; for guinea pigs, mortality is strictly dose-dependent. The incubation period for aerosol-infected animals varies greatly with the dosage, but it is usually shorter than the 2-10 (mean 4) days stated for humans. Fever is present for several days in both animals and humans. Bacteremia occurs in animals and humans but it may be intermittent and of uncertain duration. The same is true for guinea pigs infected by the peritoneal route. Leinbach et al. (1983) increased the sensitivity of detection of low levels of legionellae in naturally contaminated water samples by culturing the spleen and peritoneum of afebrile guinea pigs on the third or fourth day after inoculation. Frequently, legionellae can be isolated from the heart blood of symptomatic guinea pigs, indicating a bacteremia with possible involvement of many organ systems.

TABLE VI-1

Comparison of Natural and Experimental Legionnaires' Disease*

Humans (natural disease)	Guinea Pigs (experimental disease)
Airborne infection	Small-particle aerosol infection
Pyrexia	Pyrexia
Dyspnea	Dyspnea
Fibrinopurulent bronchopneumonia	Fibrinopurulent bronchopneumonia
Mortality (untreated), 10-20%	Mortality (dose dependent), 0-100%
Bacteria in lungs	Bacteria in lungs
Bacteremia	Bacteremia
Antibody in survivors	Antibody in survivors
Evidence of cell-mediated immunity	Evidence of cell-mediated immunity

*Source: Baskerville, 1984

A uniform distribution of macroscopic lung lesions in aerosol-infected guinea pigs is probably a reflection of uniform size of the infectious particle (Baskerville, 1984). The result is the development of a fibrinopurulent pneumonia with congestion and eventually consolidation.

The histopathology of the disease in humans and in animals is very similar. In the lungs the lesions are primarily in the alveoli. The upper respiratory tract is not affected, and the epithelium of the bronchioles is only slightly involved. Davis et al. (1983b) conducted a detailed study of the early inflammatory events in guinea pigs during experimental pneumonia produced by exposure to _L. pneumophila_ administered by the aerosol route. An analysis of bronchoalveolar lavage cells and tissue sections of the lungs indicated that the resident alveolar macrophages were the major defense mechanism in the lungs shortly after exposure. At 24-48 hours after exposure, large numbers of bacteria were associated with the macrophages and PMNs. In fact, the lavage cell population was 90% PMNs. Their appearance paralleled bacterial growth, and the number of these cells that accumulated was related directly to the size of the infecting dose. Most viable _L. pneumophila_ cells were associated with macrophages; the nonviable cells, with the PMNs. A striking observation was the essentially unrestricted growth of the bacteria -- likened to an excellent culture medium -- during the first 24 hours after infection. If during the 48- to 96-hour period after exposure the host defenses were not mobilized to check bacterial multiplication, the prognosis was grave. During this 2- to 4-day period, PMNs continued to be recruited rapidly, and toward the end of the period monocytes were evident. Destruction of the alveolar membranes released albumin, complement, clotting factors and other serum constituents into the alveolar spaces. Serum antibody was detected first at ~4 days. The immunospecific defenses together with macrophages and PMNs were mobilized to counter the infection during the postexposure period of 4-11 days.

Investigators agree that the focal lesions in the lungs in LD in both animals and humans are in the alveoli. Loss of integrity of the alveolar membranes causes an exudation of edema fluid and fibrin from the capillaries that, together with the mobilized PMNs, macrophages and necrotic epithelial cells, occludes the air sacs producing hypoxia, toxemia, respiratory failure and death (Baskerville, 1984; Davis et al., 1983c).

Intraperitoneal inoculation of guinea pigs with L. pneumophila produced a disease that in many ways is quite different from that seen after aerosol exposure (Chandler et al., 1979c; Hambleton et al., 1982). The main gross lesion was a diffuse fibrinopurulent peritonitis involving the liver and spleen. Microscopically, the peritoneum was covered by an inflammatory exudate consisting of macrophages, neutrophils, fibrin and cellular debris. From approximately the second to seventh day after infection, foci of inflammation and necrosis were regularly seen in the splenic parenchyma and often also in the liver, lungs, lymph nodes, pancreas and heart. The peritoneal exudate was rich with LD bacteria but few organisms were found in the disseminated lesions. Bacteria were found in PMNs and extracellularly, but the highest concentration was seen in the macrophages. Presumably, dissemination occurred by hematogenous or lymphatic pathways, or by a combination of the two. The guinea pig lung lesions were more focal, the interstitium was strongly involved, and necrosis and fibrin in the alveolar exudate was minimal. These histologic features of guinea pig pneumonitis differed from those seen in animals infected by the aerosol route.

As mentioned earlier in this document, guinea pigs were infected with _L. pneumophila_ by inoculating the organism into their drinking water or by gastric intubation. The disease produced was, however, a self-limited febrile illness, although _L. pneumophila_ was isolated from the lungs and spleen of some animals and some animals seroconverted (Katz and Matus, 1984). A detailed discussion of the authors' experiments and analysis of results is found in the Pathophysiologic Characteristics of Legionellae Section in Chapter IX.

Biochemical changes in legionellosis have received little attention probably because they are nonspecific, simply reflecting the effects of fever and gram-negative bacteremia and of shock (Baskerville, 1984). Hambleton et al. (1982) studied the biochemical features of guinea pigs infected by the i.p. route. Early changes included hyponatremia possibly indicating mild kidney injury, but this was not confirmed by histopathologic evidence. Striking changes occurred in serum trace metals, amino acids and proteins, resembling other acute bacterial infections of humans and animals. A decrease in serum amino acids and an increase in the ratio of phenylalanine to tyrosine possibly indicated the beginning of an inflammatory state. An increase in serum transaminase (not specified) and other liver enzymes indicated hepatic necrosis. Associated with these early biochemical changes was a leukocytosis followed by a late leukopenia.

Unlike i.p. infection, guinea pigs and other animals infected experimentally by the aerosol route seldom demonstrate extrapulmonary symptoms such as diarrhea, kidney or liver failure, or neurologic disturbances.

Winn et al. (1982) developed animal models for the study of LD using guinea pigs and rats inoculated intratracheally. Either immunologic or microbiologic evidence of infection was demonstrated in 27/28 guinea pigs and 19/20 rats that had been inoculated with 10^5-10^7 CFU of L. pneumophila. The disease in both species resembled the acute pneumonia seen in guinea pigs exposed by the aerosol route. The disease in guinea pigs was, however, much more frequently fatal, and extrapulmonary inflammatory lesions such as splenic necrosis were more common. The authors suggested that the greater resistance of rats to lethal infection provides another animal model that can be useful for studying the effect of factors such as immunosuppressive agents on susceptibility to infection by legionellae.

Attempts to infect guinea pigs with two strains of L. pneumophila by intranasal instillation of 10^9 viable organisms were unsuccessful (Fitzgeorge et al., 1983). The same two strains, however, proved fatal to the animals after i.p. inoculation of large doses. The explanation for the resistance of guinea pigs to intranasal exposure was thought to be 1) the size of the infective particles, and 2) the site of their deposition in the respiratory tract. The aerosolized infectious particles were ~5 μm in diameter, an appropriate size for reaching the alveoli, whereas the liquid deposited in the upper respiratory tract contained few such particles.

Marmosets (Callithrix jacchus) and Monkeys (Macaca mulatta). When these animals were exposed to small-particle aerosols of virulent L. pneumophila in doses of a size sufficient to produce pneumonia, the disease did not differ in any important respect from that seen in guinea pigs. In both species, an acute fibrinopurulent pneumonia was produced with extensive alveolar exudation of edema fluid and fibrin (Baskerville et al., 1983a). Rhesus monkeys were less susceptible than were guinea pigs or marmosets but this may have been due to a relatively lower infecting dose. In addition, the pulmonary lesions were less severe in monkeys than in marmosets and guinea pigs. Although bacteremia occurred in some animals, lesions were not found anywhere except in the lungs.

Rats, Mice and Gerbils. Differences in infectivity of aerosol of L. pneumophila for rats and guinea pigs were compared by Davis et al. (1983a) under similar conditions. All of the exposed 30 guinea pigs and 17 rats were infected based on microbiological and serological results, but by 7 days only 11% of the rats were dead whereas 56% of the guinea pigs had died. The illness in rats was mild, but guinea pigs had an acute pneumonia. The reasons for this difference are not clear. The disease in rats may be similar to legionellae pneumonia in humans who are in good health and who have a mild disease with a low rate of mortality.

Rolstad and Berdal (1981) studied legionellosis in rats infected by the i.p. route. They could not explain the pathogenic mechanisms responsible for death of the animals but postulated that circulatory changes involving bacterial toxins might be responsible. The short incubation period and rapid progression to a fatal result seemed to support this idea. If toxins were involved, they were heat labile or they were produced in vivo because heat-killed bacteria were not pathogenic.

Fitzgeorge et al. (1983) found Porton mice to be highly resistant to infection with L. pneumophila under the same conditions that produced infection in guinea pigs, marmosets and monkeys. The retained infective dose was progressively cleared from the lungs of Porton mice throughout a period of 7 days. The mice remained healthy throughout the observation period of 10 days, and they had not developed antibody by the end of the experiment. Patton et al. (1979) reported that ICR mice had moderate to low susceptibility to i.p. infection by legionellae but that Mongolian gerbils were highly susceptible. Arnow et al. (1982) used Mongolian gerbils successfully to isolate L. pneumophila from tapwater.

Chronic Disease

Ten days after aerosol infection by L. pneumophila and the development of pneumonia, the lungs of surviving guinea pigs were undergoing progressive organization. At the end of 1 month, areas of residual fibrosis and alveolar distortion existed but most distal air passages were cleared of debris (Baskerville et al., 1983b). At 10 days after exposure, the lesions in the lungs of rhesus monkeys were well resolved and organization was well advanced. Macrophages were invading the fibrin deposits and edema fluid was receding. Thickening of the interalveolar septa was observed and some air spaces were occluded.

At 10 and 17 days, progressive resolution and organization of exudate was occurring in the lungs of surviving marmosets infected by aerosols of L. pneumophila.

Experiments in these animals showed that alveolar fibrosis, cellular infiltration of alveolar walls, and blockage of some terminal airways were common features of the effect of L. pneumophila on the lungs at 10 days and they were still present in the guinea pigs after 1 month. The infecting organism did not persist in the lungs, and pulmonary abscesses did not develop.

The pathological changes in guinea pigs, monkeys and marmosets indicated that pulmonary fibrosis and functional impairment of the lung was characteristic of this mode of infection.

Dose Response

Huebner et al. (1984) exposed guinea pigs to a series of inhaled and retained doses ranging between 10 and 5000 CFU per animal of a Philadelphia and a Pontiac strain of L. pneumophila. Neither the strain number, the method of preparing the inoculum, nor the exposure apparatus was specified. As shown in Table VI-2, the lowest infectious dose (resulting in fever production) was 10 CFU of the Pontiac isolate and 20 CFU of the Philadelphia isolate. These are the doses that induce fever in 50% of the animals (FID_{50}). The LD_{50} was in the range of 5000 CFU. The two strains multiplied to the same extent in the lungs, and metastasized to and multiplied in the spleen at approximately the same rate. There was no significant difference in the infectivity of the Philadelphia and Pontiac strains for guinea pigs, although the former produced an acute pneumonia in humans whereas the latter produced a mild, febrile disease (Pontiac fever). The guinea pig model supplied no evidence for a disease syndrome analogous to Pontiac fever in humans.

Berendt et al. (1980) determined that the median infectious dose of the L. pneumophila Philadelphia 1 strain for male Hartley guinea pigs by the

TABLE VI-2

Dose Response of Various Animals to Experimental L. pneumophila Infection

Species/Strain	Organism	Route of Infection	Dose	Response (%)	Reference
Guinea pig/ Hartley	L. pneumophila (Burlington 1)	intratracheal	10^5 CFU	27/28 (96)^a	Winn et al., 1982
Rat/Sprague-Dawley	L. pneumophila (Burlington 1)	intratracheal	$7x10^4$-$2x10^7$ CFU	19/20 (95)^a	Winn et al., 1982
Guinea pig/ Dunkin-Hartley	L. pneumophila (74/81)	aerosol (<5 μm)	$2.4x10^3$ $3.7x10^4$ $4.9x10^5$ (inhaled-calculated)	1/12 (8.3)^b 12/12 (100)^b 12/12 (100)^b	Baskerville et al., 1983a
Monkey/rhesus	L. pneumophila (74/81)	aerosol (<5 μm)	$6x10^3$ (inhaled)	4/6 (67)^c	Baskerville et al., 1983a
Marmoset	L. pneumophila (74/81)	aerosol (<5 μm)	$2.8x10^5$ (inhaled)	3/4 (75)^c	Baskerville et al., 1983a
Guinea pig	L. pneumophila (Burlington 1)	aerosol	$1x10^3$-$5x10^5$ CFU (in lungs)	15/35 (43)^b	Davis et al., 1983b
Guinea pig/ Hartley	L. pneumophila (Burlington 1)	aerosol	$3.7x10^4$ CFU (in lungs)	9/16 (56)^b	Davis et al., 1983a
Rat/Lewis	L. pneumophila (Burlington 1)	aerosol	$1.2x10^3$ CFU (in lungs)	1/9 (11)^b	Davis et al., 1983a
Guinea pig/ Hartley	L. pneumophila (Philadelphia 1)	aerosol	$3x10^3$ CFU $2.4x10^4$ CFU $6.5x10^4$ CFU $2.3x10^5$ CFU (estimated)	0/16 (0)^b 1/16 (6)^b 4/16 (25)^b 8/8 (100)^b	Berendt et al., 1980 (LD_{50} = ~10^5 CFU)
Guinea pig/ Hartley	L. pneumophila (Philadelphia 1)	i.p.	$1.5x10^3$ $1.5x10^4$ $1.5x10^5$	0/48 (0)^b	Berendt et al., 1980

TABLE VI-2 (cont.)

Species/Strain	Organism	Route of Infection	Dose	Response (%)	Reference
Guinea pig/ Hartley	L. pneumophila (Philadelphia 1)	i.p.	1.9×10^5	1/6 (17)[b]	Berendt et al., 1980
			1.9×10^6	2/6 (33)[b]	
			1.9×10^7	5/6 (83)[b]	
			1.9×10^8	6/6 (100)[b]	
Guinea pig	L. pneumophila (Philadelphia strain)	aerosol	20	4/6[c] 0/6[b]	Huebner et al., 1984
			250	6/6[c] 0/6[b]	
			1000	6/6[c] 0/6[b]	
			5000	6/6[c] 4/6[b]	
Guinea pig	L. pneumophila (Pontiac strain)		10	4/6[c] 0/6[b]	Huebner et al., 1984
			15	6/6[c] 0/6[b]	
			500	6/6[c] 0/6[b]	
			5000	6/6[c] 3/6[b]	

[a] At least one test positive (seroconversion, culture, DFA)

[b] No. deaths/no. inoculated

[c] No. febrile/no. inoculated

aerosol route was <129 bacteria, the lowest dose tested. The 50% lethal dose was 1.4×10^5 organisms, or ~1000 times greater than the 50% infectious dose (see Table VI-2). The animals were exposed to an aerosol generated by a Collison atomizer and Henderson-type aerosol mixing and transit tube attached to a plastic exposure box. Severity of fever and serologic response were dose-related. Weight loss, fever and seroconversion were judged to be the only dependable clinical criteria of aerosol infection.

Baskerville et al. (1984) calculated a retained LD_{50} aerosol dose of $10^{4.5}$ and $10^{2.5}$, respectively, for the 74/81 and Corby strains of L. pneumophila serogroup 1 in guinea pigs. The L. pneumophila serogroup 3 strain (166/81) gave an LD_{50} of 10^4 under the same conditions.

Further discussion of the dose responses of guinea pigs and monkeys to legionellae infection is found in the Pathophysiologic Characteristics of Legionellae Section in Chapter IX.

In Table VI-2 the responses of different animal species to experimental legionellae infection by various routes are summarized. With one exception, the experiments were not designed to permit calculation of LD_{50} doses because the main objective of the investigators was to study the pathology and pathophysiology of the infected animals.

Summary

Studies of the health effects of legionellae infection in animals have involved primarily guinea pigs and rats. Guinea pigs are highly susceptible to infection by both respiratory and i.p. routes, and the disease that develops after respiratory infection is an excellent model for the study of human legionellosis. Disease in guinea pigs infected by intratracheal injection or by the aerosol route is primarily a dose-related pneumonitis or pneumonia. Somewhat less than 10^5 injected or inhaled retained cells of a virulent strain of L. pneumophila constitute the LD_{50} for guinea pigs.

Rats are more resistant to infection by legionellae aerosols, but the milder disease that develops may be a useful model in which to study the effects of immunosuppression, drugs, age and various chronic diseases on susceptibility to legionellosis.

The disease process in the lungs of susceptible animals is characterized by the multiplication of Legionella in recruited macrophages with destruction of the macrophages and release of toxic products. The membrane integrity of the alveoli is destroyed, and serum proteins together with PMNs, macrophages, bacteria and debris fill the air sacs producing hypoxia and impairing respiratory function.

Monkeys and marmosets can be infected with L. pneumophila by the aerosol route, but the disease is generally milder than in guinea pigs and is more like that seen in rats. Gerbils are highly susceptible to infection by the i.p. route.

VII. HEALTH EFFECTS IN HUMANS

Acute Disease

Attack Rate. The attack rate in epidemic legionellosis is shown in Table III-5 for 17 outbreaks of LD and two outbreaks of Pontiac fever. The rates varied from <0.3-13% for LD and from 95-100% for Pontiac fever (England, 1981). Attack rates in nosocomial LD and estimates of the prevalence of sporadic LD were discussed in the Incidence: Outbreaks and Sporadic Cases Section in Chapter III.

Incubation Period. The incubation period of LD during the Philadelphia outbreak in 1976 was determined to be 2-10 days with a mean of 5 days (Fraser et al., 1977). In eight additional LD outbreaks for which incubation periods were estimated, the range was 1-18 days (Cordes and Fraser, 1980). Myerowitz et al. (1979) noted that opportunistic lung infections with *L. micdadei* occurred in hospital patients within 6-21 days (mean 14 days) following administration of high-dose corticosteroid therapy.

The incubation periods for Pontiac fever in the Pontiac and James River, VA, outbreaks were 5-66 hours (median 36 hours) and 17-43 hours (median 37 hours), respectively (Cordes and Fraser, 1980). A recent outbreak of Pontiac fever that affected women using a whirlpool spa had an incubation period of 3-38 hours (Remis, 1983). A single case in Vermont in which the exact time of exposure was known had an incubation period of 46 hours (Girod et al., 1982).

Duration of Illness. The duration of illness in victims of fatal pneumonia during four outbreaks of LD is shown in Table VII-1, the range

TABLE VII-1

Duration of Illness of Cases of Fatal Pneumonia Caused by *L. pneumophila*[a]

Source	No. Cases	Immunologic Status	Duration of Illness (days)	
			Range	Average
Philadelphia[b]	26	Presumed immunocompetent	4-21	8
Vermont[c]	14	Immunocompromised	5-16	8
Spain (Scottish series)[d]	5	Presumed immunocompetent	8-13	9
Wadsworth[e] VA Hospital	20	Presumed immunocompetent and immunocompromised	2-21	NR

[a] Source: Blackmon et al., 1981

[b] Blackmon et al., 1978

[c] Winn et al., 1978

[d] Boyd et al., 1978

[e] Hernandez et al., 1980

NR = Not reported

being 2-21 days with an average of ~8 days. Fever continues until the patient receives appropriate antimicrobial therapy or until the infection is spontaneously resolved on the 7th to 11th day. Patients may have persistent fatigue, weakness and faulty memory for months after an acute illness (Meyer, 1983). The recovery of patients who survive LD pneumonia depends upon so many variables that it is difficult to predict the length of the recovery period. Dysmetria, ataxia, dysarthria and diminished deep-tendon reflexes are sometimes observed. Renal insufficiency usually is resolved within weeks or months but dialysis may be required. Patients with respiratory failure during the acute episode may develop diminished respiratory capacity, and fibrosis may result from septal injury (Meyer, 1983). The physical condition of the patient, age, accuracy of diagnosis and adequacy of therapy are important factors in determining length of convalescence.

Pontiac fever, in contrast to LD, is a self-limited illness usually lasting 2-5 days (Fraser et al., 1979; Glick et al., 1978). No deaths or long-term sequelae have been reported for this disease.

Symptoms. The major symptoms of LD are shown in Table VII-2 for patients in the Philadelphia outbreak of 1976 and for nosocomially acquired cases at the Wadsworth VA Medical Center (Tsai et al., 1979; Kirby et al., 1980). Clinical and laboratory parameters in LD were compared with non-legionnaires' pneumonia by Yu et al. (1982). Hyponatremia (low serum sodium) was the only clinical parameter that was significantly different ($p<0.0001$) for LD patients. Kirby et al. (1980) also reported low sodium levels in 54% of their patients. Other symptoms and signs that are strongly associated with LD are high fever and neurologic manifestations of confusion

TABLE VII-2

Symptoms of Legionnaires' Disease

Symptom	Number of Patients[a]	Percent Patients	Symptom	Number of Patients[b,c]	Percent Patients
Cough	61	92	Fever	118	97
Chills	61	77	Malaise	86	89
Diarrhea	61	47	Cough	96	86
Dyspnea	61	36	Chills	70	74
Chest pain	61	33	Dyspnea	50	59
Headache	61	28	Myalgias	47	55
Sweats	61	26	Headache	49	53
Nausea, vomiting	61	25	Chest pain	46	52
Myalgia, arthralgia	61	20	Sputum production	47	50
Abdominal pain	61	0	Purulent sputum	23	49
Fever ≥38.9°C	63	90	Diarrhea	41	41
Relative bradycardia	48	60	Vomiting	22	23
Neurologic findings	61	38	Abdominal pain	17	20

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[a] Patients evaluated at Wadsworth Medical Center. Source: Kirby et al., 1980

[b] Source: Tsai et al., 1979

[c] Patients evaluated having the symptom. Not all patients were evaluated for all symptoms. Patient total was 123. Philadelphia outbreak, 1976.

and encephalopathy; also, elevated serum transaminase levels occurred with significantly ($p<0.05$) greater frequency in LD patients than in patients with pneumococcal or mycoplasmal pneumonia (Helms et al., 1979). In a study of 24 cases at Wadsworth Medical Center, the association of otherwise unexplained diarrhea with febrile respiratory illness strongly suggested a diagnosis of LD (Kirby et al., 1978).

Laboratory findings in LD at Wadsworth Medical Center are shown in Table VII-3 (Kirby et al., 1980). With the exception of low serum sodium levels, these characteristics did not usually assist in distinguishing Legionella pneumonia from pneumonias of other etiologies. Band and Fraser (1981) included LD in their tabular listing of the comparative epidemiologic and clinical features of eight of the atypical pneumonias.

Radiographically, Legionella pneumonia initially presents a picture of acute lung infiltrates. These were usually unilateral, patchy, segmented or lobar alveolar infiltrates, although 5-30% of the patients initially had bilateral disease (Broome, 1983). Eventually, 25-65% of the patients developed bilateral involvement as the disease progressed rapidly over the first 3-5 days. These infiltrates were not specific for LD (Broome, 1983). Cavitations have been reported in some cases of LD, and pleural effusions were noted in 7-63% of cases (Broome, 1983; Gump et al., 1979; Saravolatz et al., 1979a). Cavitary disease is more likely to occur in immunocompromised persons. In general, radiographic abnormalities persisted for 1 or more months after an acute illness (Broome, 1983).

TABLE VII-3

Clinical Laboratory Data on Patients with Legionnaires' Disease at Wadsworth VA Hospital[a]

Data	Percent Having the Characteristic
Leukocytosis	78 (45/58)[b]
Hyponatremia	54 (30/55)
Hypophosphatemia	51 (22/43)
Elevated lactic dehydrogenase	88 (36/41)
Elevated serum glutamic oxaloacetic transaminase	49 (21/43)
Elevated alkaline phosphatase	49 (20/41)
Elevated bilirubin	15 (7/47)
Proteinuria	45 (15/33)
Hematuria	None
Renal failure	None

[a] Source: Kirby et al., 1980

[b] No. with characteristic/no. evaluated

Many clinicians think that LD cannot be easily distinguished by clinical and laboratory characteristics from pneumonia caused by other agents (Yu et al., 1982; Herwaldt and Fraser, 1982). Storch and Avioli (1982) stated that no one finding was by itself specific. This was particularly true for radiologic and laboratory findings (Saravolatz et al., 1979a; Helms et al., 1981). Kirby et al. (1978) stated, however, that although no single aspect of LD was unique, the overall clinical presentation was often distinctive enough to furnish an early, almost certain, diagnosis. They thought that the association of an otherwise unexplained diarrhea with febrile respiratory illness was a good indicator of LD.

The exact nature of the relationship between LD and Pontiac fever is not known. In the Pontiac outbreak of 1968 the entire symptom complex appeared within 6-12 hours of onset (Glick et al. 1978). In some patients the illness began abruptly with fever and shaking chills. More often progressive malaise, diffuse myalgia and headache were noted. Muscle pains, dizziness, cough and nausea were common symptoms. Diarrhea occurred in 15% of the patients, 33% experienced chest pain and 57% had a dry cough. Some had a variety of mild neurological symptoms. The blood chemistries of some patients were slightly abnormal, and some of the seven acutely ill persons had elevated white cell counts. In short, except for the virtual absence of upper and lower respiratory tract disease, the illness resembled a mild form of LD.

The symptom complex in 10 men who developed Pontiac fever after cleaning a steam turbine condenser was very similar to that seen in the Pontiac patients (Fraser et al., 1979). Although nine of the men were hospitalized, none had pneumonia or objective signs of other organ system involvement.

One of two men receiving common source exposure to cooling tower spray contaminated with L. pneumophila serogroup 1 developed typical mild Pontiac fever, whereas the other man had a very severe pneumonia and was hospitalized for 79 days (Girod et al., 1982). Of interest was the fact that both men seroconverted with IFA titers of 1024 or greater.

Fourteen women who were exposed to a whirlpool contaminated with L. pneumophila serogroup 6 had symptoms of fever, chills, chest pain, cough and nausea, the severity of the symptoms increasing with the time spent in the whirlpool (Remis et al., 1983). Eleven of the women had seroconversions to L. pneumophila serogroup 6 antigen. This was the first report of Pontiac fever caused by a serogroup of L. pneumophila other than serogroup 1.

A new species of Legionella (L. feeleii) caused an explosive outbreak of Pontiac fever affecting 395 engine assembly plant employees (Herwaldt et al., 1984). The organism was isolated from an oil-based coolant. Antibody titers were significantly higher in ill employees than in controls ($p=0.0001$), and attack rates decreased from 100% to 8% with increasing distance from the contaminated coolant system. This is the first report of Pontiac fever caused by a species other than L. pneumophila.

Pathology. In one of the first pathological studies of LD, Blackmon et al. (1978) presented their findings in 26 cases from the 1976 Philadelphia epidemic. These included lung weights, gross macroscopic appearance, presence or absence of pleural effusions and adhesions, microscopic findings and other autopsy findings. As was the case with guinea pigs experimentally infected by Legionella aerosols, the consistent pathological changes in humans were restricted to the lungs.

Winn et al. (1982) made an interesting comparison of Legionella pneumonia in humans, guinea pigs and rats (Table VII-4).

Gross Pathology -- In LD the lung commonly has the characteristics of a confluent lobular pneumonia (bronchopneumonia) although it has also been described as lobar (Winn et al., 1982; Blackmon et al., 1981). The lobular infiltrates were sometimes so extensive that they involved most of one or more lobes. The pathologic appearance agreed well with radiologic description of diffuse, fluffy, alveolar infiltrates that eventually involved large portions of a lobe (Winn, 1982). Radiologists suggested that septic pulmonary infarcts might induce the pleural-based infiltrates that were often seen in gross examinations. Gross findings typically included congestion and areas of consolidation, the latter suggesting lobar pneumonia. When the lung was sliced, however, the lesions were usually seen to be associated with the bronchioles. Usually, inflammation did not extend across the lobar septa. Significant hemorrhage in the lung parenchyma was uncommon. Abscess formation and cavitary disease were also uncommon. Pleural effusions and adhesions have been observed frequently at the time of autopsy. In many cases the pneumonia was bilateral without any pronounced tendency to affect any particular lobe or segment within lobes.

TABLE VII-4

Comparison of *Legionella* Pneumonia in Humans, Guinea Pigs and Rats[a,b]

	Human	Guinea Pig	Rat
Clinical			
Fever	+++	+++	0
Labored respirations	+++	++	+
Microbiologic			
Recovery of *Legionella* from lungs during acute pneumonia	+++	+++	+++
Immunologic			
Seroconversion	+++	+++	+++
Demonstration of *Legionella* in lungs	+++	+++	+++
Demonstration of *Legionella* in extrapulmonary organs	+ to ++	+++	+
Pulmonary pathology			
Confluent macroscopic pneumonia	+++	+++	++
Macroscopic abscess	+	0	0
Airspace infiltrate of neutrophils and macrophages	+++	+++	+++
Leukocytolysis	++	+	+
Reactive interstitium	++	++	++
Hyaline membranes	++	±	±
Extrapulmonary pathology			
Splenic necrosis	±	+++	±
Hepatic sinusoidal macrophages	±	++	0

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[a] Source: Winn et al., 1982

[b] 0 to +++ = frequency of occurrence and/or extent of lesion: 0 = not observed; ± = <5%; + = 5-30%; ++ = 31-70%; +++ = 71-100%

The cut surfaces of consolidated areas of the lungs of LD victims were reddish gray, friable, moist, granular and airless. The visceral pleura was frequently covered with a thin fibrinous exudate. After fixation, the granular surface of the lung could be scraped to obtain tissue for smear preparation and for rapid diagnosis by DFA staining procedures (Cherry et al., 1978). During the study of 26 of the well-defined LD cases from the Philadelphia outbreak, the Special Expert Pathology Panel noted some pathologic changes in organs other than the lungs but none of these was consistently associated with LD. Extrathoracic localization of *Legionella* in other organ systems, usually without associated pathological changes, has been reported (Evans and Winn, 1981; Hicklin et al., 1980; Watts et al., 1980).

Histopathologic (Microscopic) Features -- The microscopic pathology of legionellae in human lung tissue is very similar to that described for the guinea pig model infected by the respiratory route and discussed in Chapter VI, Health Effects in Animals. The disease is an acute fibrinopurulent pneumonia sometimes associated with acute, diffuse alveolar injury. The microscopic findings for 20 fatal cases were tabulated by Hernandez et al. (1980) and are presented in Table VII-5. Although not specified, *L. pneumophila* was apparently the etiologic agent in these 20 cases. A larger series of pneumonia cases caused by *L. pneumophila*, together with a number of cases caused by *L. micdadei*, *L. bozemanii* and *L. dumoffii*, were studied by Winn and Myerowitz (1981). The microscopic features of these pneumonias are shown in Table VII-6. Acute alveolitis and bronchiolitis were histologically the most important pulmonary lesions. The alveolar exudate consisted of a mixture of macrophages, PMNs, red blood cells and proteinaceous

TABLE VII-5

Microscopic Findings in 20 Fatal Cases of Legionnaires' Disease*

Pathology	Number of Cases
Intra-alveolar exudate	
Predominantly fibrinous	3
Mixed macrophages: polymorphonuclear leukocytes (3:1)	7
Mixed polymorphonuclear leukocytes: macrophages (5:1)	8
Lysis of infiltrate	7
Coagulation necrosis	7
Hyaline membranes	4
Septal edema	7
Vascular thrombosis	4
Interstitial inflammation	2
Vasculitis, nonspecific	1
Hemorrhagic component	6
Bronchitis and bronchiolitis	14
Intra-alveolar organization	2
Diffuse fibrosis	1

*Source: Hernandez et al., 1980

TABLE VII-6

Microscopic Features of the Legionella Pneumonias*

		L. micdadei		L. bozemanii
	L. pneumophila	Biopsy	Autopsy	L. dumoffii
	(n=53)	(n=7)	(n=7)	(n=4)
Infiltrate				
Neutrophils predominant	12	7	4	1
Neutrophils and macrophages	30	0	2	0
Macrophages predominant	9	0	1	0
Indeterminate	2	-	-	3
Leukocytoclasis	33	3	6	3
Small vessel "vasculitis" or thrombosis	16	2	2	3
Coagulative necrosis	6	0	0	0
Septal necrosis	20	0	5	3
Remote alveolar damage	13	0	1	0

*Source: Winn and Myerowitz, 1981

material. Lysis of the cells of the alveolar exudate was common. Microscopic abscesses and coagulative necrosis of lung tissue was observed in some cases. The lung interstitium was sometimes inflamed. The alveoli were usually the site of most of the inflammatory exudate, but small bronchioles and occasionally larger bronchioles or bronchi were plugged with cellular debris (Winn and Myerowitz, 1981).

When appropriate stains were used, large numbers of legionellae cells were seen to be intracellular in PMNs and macrophages as well as extracellular in the lysed inflammatory exudate of the alveoli. Staining reactions of four species of legionellae in both smears and tissue sections were summarized by Winn and Myerowitz (1981). It was found that _L_. _micdadei_ and, rarely, other species may be acid-fast with some tissue stains. The silver impregnation stain of Dieterle, although technically difficult and nonspecific, is an excellent stain for demonstrating legionellae in tissue sections because it stains the organisms very well (Blackmon et al., 1981). The DFA staining of smears or tissue sections provides a specific and rapid means of detecting legionellae.

Ultramicroscopically, the legionellae were found within membrane-bound structures of macrophages known as phagosomes. The bacteria appeared to be associated with membranes that are bounded by ribosomes (Winn, 1982). Large numbers of _Legionella_ were found intracellularly within macrophages but also were seen within PMNs. Often the bacteria were seen to be in the process of dividing by a nonseptate pinching process. A three-layered inner membrane and outer cell wall, dispersed nuclear material, electron-lucent vacuoles, ribosomes and, depending upon the material and its preparation, a peptidoglycan layer were characteristic of this gram-negative intracellular bacterium (Winn and Myerowitz, 1981; Chandler et al., 1979b; Winn, 1982).

Extrapulmonary Effects -- Extrapulmonary effects of LD have been described and related to the hematogeneous or lymphatic distribution of the organisms and to poorly-defined toxic effects. Congestion and enlargement of the spleen and liver, cardiomegaly, reactive lymph nodes and occasional kidney pathology have been attributed to extensions of the pneumonic disease. Legionella cells and aggregates of soluble antigen have been demonstrated by DFA staining in some of these sites. Generally, however, manifestations of extrapulmonary infection were considered to represent disseminated toxic effects of pulmonary disease (Winn and Myerowitz, 1981; Blackmon et al., 1981).

Chronic Disease

The chronic effects of LD were difficult to evaluate because of the inability to demonstrate the bacteria in tissues following the acute phase and because mixed infections or preexisting tissue injury clouded the interpretation of the observations (Winn and Myerowitz, 1981; Blackmon et al., 1981). Most patients recovered from LD without serious pulmonary pathology. According to Lattimer et al. (1979), however, some of the survivors of the Philadelphia outbreak had a significant decrease in lung diffusing capacity fully 2 years after the acute infection. In some patients interstitial and intra-alveolar fibrosis, interstitial inflammation, desquamation of alveolar linings, obliteration of respiratory bronchioles and alveolar ducts, chronic vasculitis and chronic organizing pleuritis were observed (Blackmon et al., 1981).

A carrier state has not been documented for the legionellae. Saravolatz et al. (1984), however, found that the oropharynx of 2/70 (2.9%) renal transplant recipients and 3/62 (4.8%) hemodialysis patients were colonized with *Legionella*-like organisms as identified by DFA staining. Colonization occurred only with *L. micdadei* and *L. dumoffii* and lasted for 2-24 weeks. These results are questionable because no legionellae were isolated and none of the patients developed pneumonia or had a rise in antibody titers. Very little is known about cross-reactions between *L. micdadei* and *L. dumoffii* and non-*Legionella* bacteria, but *L. micdadei* has been reported to cross-react with a *Pseudomonas maltophila* culture isolated from a tracheal swab of a patient with pulmonary edema (Orrison et al., 1983a). Establishment of a carrier state for legionellae requires better data before it can be substantiated.

The rare chronic effects noted by Pontiac disease patients were either nonspecific or could not be significantly related to the acute episodes (Glick et al., 1978; Fraser et al., 1979; Remis et al., 1983).

Mortality Rates

The mortality rates calculated for patients in 17 outbreaks of LD and 2 outbreaks of Pontiac fever are shown in Table III-5. Mortality rates were discussed in the Specific Occurrence Section in Chapter III. The case fatality ratio in 15 of the earlier outbreaks of LD listed by Broome (1983) ranged from 14-34%; the case fatality ratio in patients treated with erythromycin was 0-11%. Herwaldt and Fraser (1982) listed a case fatality ratio of 5-40%, apparently applying to combined treated and untreated cases.

Mortality is high in immunosuppressed patients; 5/8 such patients died of pneumonia caused by the Pittsburgh pneumonia agent, *L. micdadei* (Myerowitz et al., 1979). In another series of 64 patients, 80% of the immunosuppressed patients not receiving erythromycin died in comparison with 24% receiving the drug (Kirby et al., 1980). Only 7% of nonimmunosuppressed patients receiving erythromycin died, whereas 25% of them died when the drug was not used. For erythromycin therapy overall, only 13% died, whereas 55% of those not so treated died. The case fatality rate for the 64 patients was 25%. In a series of 71 nosocomial cases of LD reported by England and Fraser (1981), 31% died of the infection. In a study of 1005 cases of sporadic legionellosis studied by England et al. (1981), 19% were fatal.

Dose Response

Data bearing directly on dose response for human legionellosis are not available. The requirements for obtaining meaningful information are as follows: 1) exposure of human subjects to aerosols or to ingestion of a known volume of a known concentration of viable or nonviable legionellae, 2) estimation of the dose received based on time of exposure and respiratory volume, 3) knowledge and control of aerosol particle size and 4) quantitation of the dose actually reaching the lungs. Obviously, these conditions could not be met except by exposure of human volunteers.

Although the guinea pig is very good model for the study of airborne legionellosis, data from animal studies cannot be extrapolated to the human condition. Even if all experimental parameters were closely controlled, human dose response to infection with legionellae would be more variable than that for animals because of a variety of risk factors that are difficult to control, not the least of which is genetic heterogeneity.

Quantitative studies of human exposure to legionellae have not been reported. Two maintenance workers were exposed to cooling tower mist containing Legionella when the fan was accidentally turned on. One developed acute LD pneumonia whereas the other, less exposed individual developed Pontiac fever (Girod et al., 1982). There was a suggestion in one recurrent hotel outbreak of LD that persons who were the first to wash in the morning were more likely to contract LD, presumably because they received a larger infective dose of the bacteria (Bartlett et al., 1984). In another study there was a significant association between illness and distance from hospital room to the showers, suggesting a difference in the size of aerosol doses of LDB that were received. Hyperchlorination may release large amounts of scale and minerals from the plumbing system. Increased numbers of LDB in the water and outbreaks or exacerbations of endemic LD have followed such procedures. Legionella have been isolated from ice machines (Yee, 1984). Quantitative measures of legionellae in aerosols concurrent with ongoing outbreaks or in endemic nosocomial situations are badly needed.

Modes of Transmission

Many investigators have expressed their assumptions and frustrations about the mode of transmission of legionellosis, most without acceptable proof of the exact mechanism. The specific occurrences that suggested possible modes of transmission were detailed in the Specific Occurrence Section in Chapter III. Direct proof of a given mode would require a series of experimentally documented steps similar to those described in the Dose Response Section of this chapter. The incident in which two men were infected when a cooling tower on which they were working was accidentally turned on is a good example of cause and effect (Girod et al., 1982).

Another good example of a mode of transmission was that of Zuraveleff et al. (1983), who demonstrated that a naturally contaminated hospital humidifier produced aerosols that gave colonies of L. pneumophila serogroup 1 on exposed culture plates and caused seroconversion in sentinel guinea pigs. Possible reservoirs of transmission and key references to studies or observations relating to them are given in Table VII-7. Unfortunately, acceptable epidemiologic data implicating these reservoirs as the cause of human infection are not available for most of those listed.

Finally, it should be remembered that sporadic cases of LD have usually not been associated with air-conditioning systems (Storch et al., 1979b). Also, some outbreaks of LD occurred at sites without air-conditioning (Thacker et al., 1978; Reid et al., 1978).

Immunity

Some facets of immunity to legionellae were discussed in the Virulence Section in Chapter II.

L. pneumophila and some other species of legionellae have been shown to be facultative IC pathogens (Horwitz and Silverstein, 1980, 1981a,b,c). Large aggregrates of Legionella bacteria positioned intracellularly in macrophages were observed in the lungs of fatal cases of LD (Chandler et al., 1979a; Blackmon et al., 1978; Winn et al., 1978). The bacteria also were found intracellularly in PMNs and extracellularly in the alveoli with fibrin and cellular debris.

TABLE VII-7

Summary of Possible Reservoirs of Transmission of Legionella

Mode	Key References
1. Aerosols of water (inhalation)	
a. Heat-rejection equipment • Cooling towers • Evaporative condensers • Steam turbine cleaning	Dondero et al., 1980; Klaucke et al., 1984; Band et al., 1981; Glick et al., 1978; Cordes et al., 1980a; Fraser et al., 1979
b. Showers and faucets (hot and cold water) • Hot water tanks	Tobin et al., 1980, 1981a,b; Stout et al., 1982; Shands et al., 1980; Fisher-Hoch et al., 1981a; Colbourne et al., 1984a,b; Ciesielski et al., 1984; Meenhorst et al., 1981; Cordes et al., 1981; Wadowsky et al., 1982; Helms et al., 1983; Yee and Wadowsky, 1982
c. Nebulizers d. Humidifiers e. Whirlpool spas f. Fountains (malls)	Gorman et al., 1980; Jones et al., 1984; Arnow et al., 1982; Zuravleff et al., 1983; Witherell et al., 1983; Cherry and Orrison (1979)
2. Ingestion of Water	
a. Potable	Fraser et al., 1977; Yu et al., 1981; Saravolatz et al., 1984; Katz and Matus, 1984; Dournon et al., 1982; Edelstein et al., 1982b; Desplaces et al., 1984
b. Raw (immersion)	Thomason et al., 1979a
3. Aerosol of mixtures (oil and water)	Shands, 1984
4. Excavations, dust, soil	Thacker et al., 1978; Marks et al., 1979; Conwill et al., 1982

In a brilliant series of experiments, Horwitz and Silverstein (1980, 1981a,b,c) showed that L. pneumophila multiplies intracellularly in human monocytes *in vitro* but not in lymphocytes or in PMNs. The doubling time for IC growth was <2 hours, equal to or better than that reported for the best artificial media. Intact, viable monocytes in direct contact with the bacteria were required under all conditions in order to obtain intracellular growth of the Legionella. Similar findings were reported for the interaction between normal cynomolgus monkey alveolar macrophages and LDB (Kishimoto et al., 1979). The bacteria were ingested, multiplied, and then destroyed these phagocytic cells.

The phagocytized bacteria were located in membrane-bound cytoplasmic vacuoles that were surrounded by many small particles resembling monocyte ribosomes (Horwitz, 1983). The function of both the vacuoles and the particles is unknown. Similar features have been observed in alveolar macrophages from the lungs of LD victims (Glavin et al., 1979).

In another experiment it was shown that antibody and complement promoted phagocytosis of L. pneumophila by PMNs but that few of the opsonized bacteria were killed (Horwitz and Silverstein, 1981b). This observation suggested that the PMNs were not a decisive factor in protecting the host against L. pneumophila. Monocytes exposed to complement and antibody efficiently phagocytized the LDB but, again, only a small number (0.25 log) of the bacteria were killed. Monocytes bind more than 3 times as many viable L. pneumophila cells in the presence of both antibody and complement as they do in the presence of complement alone, and both reactants are necessary for any killing effect (Horwitz and Silverstein, 1981b). Once internalized, however, the surviving bacteria multiply as rapidly as they do when serum is omitted. These observations with monocytes also suggested that humoral immunity dependent upon antibody and complement may have little beneficial effect in protecting a host against infection with Legionella.

In other experiments it was demonstrated that human monocytes could be activated to inhibit the IC multiplication of L. pneumophila (Horwitz and Silverstein, 1981c; Horwitz, 1983). This was done by incubating the monocytes with the mitogen concanavalin A (Con A) and lymphocytes. The monocytes were then activated to inhibit multiplication of Legionella. The process was dependent upon stimulation by the lymphocytes. It was determined, however, that cell-free supernatants from Con-A-lymphocyte-activated monocytes also were effective in inducing other monocytes to inhibit multiplication of Legionella. The effect was proportional to the concentration of supernatant added. Supernatant-activated monocytes inhibited multiplication of the bacteria by limiting the number of phagocytized L. pneumophila cells to ~50% the number phagocytized by nonactivated cells. In addition, the doubling time of internalized bacteria was 3.1 times longer than in nonactivated monocytes (Horwitz, 1983).

Recently, Horwitz (1983) examined blood mononuclear cells from patients recovered from LD to determine if they had characteristics compatible with cell-mediated immunity. He found that these cells were activated by L. pneumophila to produce supernatants that were able to activate normal monocytes to inhibit L. pneumophila multiplication.

The question of specific protection against infection was addressed by Wong and Feeley (1983) in experiments in which guinea pigs were immunized without various serogroup antigens of L. pneumophila. The animals were challenged by i.p. injections of virulent strains after ~2 weeks and after 11-14 months. Protection (survival) after challenge was primarily serogroup specific. At 11-14 months it was ~100%; cross protection (heterologous serogroups of L. pneumophila) lasted only a few weeks. To some extent the serogroup-specific immunity could be passively transferred with immune IgG, but other factors were involved. The serogroup antigen was a very effective adjuvant in potentiating antibody and in inducing delayed hypersensitivity (Wong and Feeley, 1983).

Baskerville et al. (1983c) attempted to protect guinea pigs against lethal aerosol challenge with L. pneumophila by exposing them to 1, 2 or 3 sublethal aerosol infections. The animals developed serum antibodies after each infection but were not protected against the lethal (10 LD_{50}) challenge dose. In fact, the animals previously exposed died much sooner than the nonimmunized controls even though their lung bacterial counts were lower. The severity of pulmonary lesions increased after each successive sublethal injection. Animals immunized with serogroup-specific antigen extracted from the bacterial cells also developed antibodies but were not protected against lethal challenge and they also died earlier. One unproven explanation offered for these findings was the possibility that bactericidal antibody activity reduced the number of bacteria in the lungs but increased complex interactions between bacterial products and host defenses, thereby exacerbating tissue damage. These experiments emphasized once again the undefined relationship between humoral and cell-mediated immunity. As pointed out above, both serogroup-specific and species-specific antibody play a role in immunity against Legionella, but whether or not this role is beneficial or harmful to the host animal may depend upon other factors. The route of immunization, the route of challenge, the size and timing of the immunizing and challenging doses and the existing state of cell-mediated immunity in the host may determine the outcome of infection. The relative importance of these various aspects of immunity have not been clarified.

Summary

A strange and unexplained facet of legionellosis is the striking difference in the attack rates in epidemic LD compared with those for Pontiac fever. The incubation period and duration of illness in Pontiac fever is, on the average, shorter than that for legionellosis. LD is typically an acute fibrinopurulent bronchopneumonia, whereas Pontiac fever is a mild self-limited disease with the virtual absence of upper and lower respiratory tract disease.

L. pneumophila is a facultative intracellular parasite that localizes in the alveoli and terminal bronchioles of the lung where it is phagocytized by both PMNs and by the resident and mobilized macrophages. A few bacterial cells are killed by the PMNs, but the major events occur in the macrophages which, after killing a small proportion of the bacteria, support intracellular growth of the remainder that is equal to growth in the best culture media.

The process results in destruction of the phagocytic cells, in breaching the integrity of the alveolar membranes, and in plugging the air sacs with PMNs, macrophages, erythrocytes and fibrin. The result is a lobular or lobar pneumonia with accompanying severe respiratory insufficiency and certain poorly understood extrapulmonary effects.

Most LD patients recover without serious pulmonary pathology; some develop fibrosis, interstitial inflammation and other chronic conditions. The case fatality rate in 15 outbreaks ranged from 14-34%; in patients treated with erythromycin, 0-11%.

The weight of evidence derived from both _in vivo_ and _in vitro_ studies in animals and in humans appears to be that cell-mediated immunity is the key to protection against infection with _L. pneumophila_ and, perhaps, also _L. micdadei_. The interaction of _Legionella_ with host PMNs and monocytes, skin-test reactivity in experimental infection, lymphocyte transformation in patients recovered from LD, and the efficacy of antimicrobial drugs such as erythromycin and rifampin that can penetrate macrophages constitute evidence for cell-mediated immunity.

The importance in the pathogenesis of legionellosis of humoral immunity and of a variety of toxic factors produced by the bacteria is largely unknown.

Related to the observations that resistance to infection by legionellae is mediated primarily by cellular immune mechanisms, it is not surprising that patients with diseases in which immunosuppression is a component are generally those having the highest relative risk of infection by legionellae and the highest mortality (see Tables IX-1, IX-3 and IX-4). This cohort of patients (those receiving renal hemodialysis or undergoing transplantation, those with cancer, and those receiving immunosuppressive drugs) are also those most likely to be hospitalized and subject to nosocomial infections with legionellae.

Circumstantial data indicate strongly that human legionellosis occurs primarily by transmission of the bacteria from water sources (reservoirs) to the human lungs by means of aerosols. Satisfactory epidemiologic validation of this assumption has been reported in relatively few cases. A major problem is the absence of experimental data confirming the presence, viability, virulence and concentration of airborne legionellae in an appropriate temporal and spatial relationship to the population at risk.

Reservoirs that have been closely linked to aerosol infection are heat-rejection equipment, hot water tanks, hot water from showers and faucets, nebulizers and humidifiers.

Tantalizing indications, but no hard data, exist for infection by means of contaminated water. The possibility that legionellae are aspirated into the lungs following colonization of the oropharynx also deserves further investigation.

VIII. SYMPTOMS AND MECHANISM OF DISEASE PRODUCTION

The mechanism of disease production by legionellae is not completely understood either in the lungs or in extrapulmonary infection (Wong and Feeley, 1983; Winn and Chandler, 1982). The role of various toxic factors was discussed in the Virulence Section in Chapter II, and the pathology of the disease in animals and in humans was reviewed in Chapters VI and VII, respectively.

Symptoms associated with the pulmonary phase of LD are listed in Table VIII-1 together with the mechanisms that are known or have been proposed to explain them. The endotoxin of legionellae is atypical of that seen in other gram-negative bacteria primarily because it is relatively low in toxicity to animals, in pyrogenicity, and in inducing the Swartzman reaction (Wong and Feeley, 1983). Its low pyrogenicity is interesting in view of the acute febrile reactions of LD patients. The group-specific antigens are included in the endotoxin complex, and they are quite antigenic and have superior adjuvant characteristics.

The other major disease-producing mechanism of legionellae is activated when the bacteria reach the terminal bronchioles and the alveoli of the lungs. The lungs appear to be the major, perhaps many times, the only target organ for legionellae. It is here that a complex series of interactions occur, the outcome of which determines the degree of the disease process, if any. If the bacteria are successful in thwarting the protective phagocytic mechanisms of the PMNs and macrophages, they proliferate intracellularly in the macrophages. The result may be extensive tissue damage and the development of an acute fibrinopurulent lobular or lobar pneumonia.

TABLE VIII-1

Mechanisms of Disease by Legionellae

Symptom	Mechanism	Reference
Headache, fever, granulocytosis, thrombocytopenia, vasoconstriction, weight loss, lethality	Low toxicity endotoxins (LPS) = serogroup antigens = pyrogens	Wong and Feeley, 1983
Schwartzman reaction, antibody response	Serogroup-specific antigens with adjuvant characteristics	Wong and Feeley, 1983
Intravascular coagulation	Endotoxin stimulates procoagulant activity	Fumarola et al., 1984; Dowling et al., 1983
Shock	Endotoxins	Dowling et al., 1983
Suppression of phagocytosis	Cytotoxin (peptides) impairs O_2-dependent sequelae of phagocytosis in PMNs	Friedman et al., 1980, 1982
Lowered nonspecific resistance (antiprotease deficiency in the lung)	Degradation of human serum proteins including acute phase and ß-lipoprotein	Muller, 1980
Hemoptysis and injury to leukocytes and tissue cells	Exotoxin with phospholipase C (lecithinase) activity	Baine, 1984
Cough, chest pain, dyspnea, hypoxemia, tissue damage in lung (fibrinopurulent bronchopneumonia), respiratory failure	Lysis of *Legionella*-infected PMNs and macrophages by proteolytic enzymes with release of toxic substances of host and bacterial origins. Exudation of fibrin from injured alveolar membranes. Acute diffuse alveolar damage. Coagulative necrosis. Occlusion of air sacs.	Blackmon et al., 1978; Winn and Myerowitz, 1981

During the development of the pneumonia, the patient experiences the symptoms of chest pain, cough, dyspnea, hypoxemia, and eventually, if extensive enough, respiratory failure. The studies of Horwitz and Horwitz and Silverstein summarized by Horwitz (1983) with in vitro human monocytes have illuminated the steps in the process that determine the outcome of the host cell-parasite interaction. These have been discussed in the Immunity Section in Chapter VII.

IX. QUANTIFICATION OF HEALTH EFFECTS

Potential Exposure of the U.S. Population to Legionellosis

Potentially, the entire U.S. population is at risk of infection by legionellae because 1) the bacterium is ubiquitous in the aqueous environment and perhaps in soil; 2) the exact sources of infection are in most cases unknown; 3) the relative importance of the suspected sources in inducing infection has not been clarified; and 4) the factors controlling human susceptibility are not well understood.

Legionellosis includes LD and Pontiac fever because both are caused by bacteria belonging to the genus Legionella. Pontiac fever is a mild, febrile, self-limited disease with a high attack rate but without mortality. Only a fairly small number of cases (~500) have been reported. Legionnaires' disease is an acute lower respiratory tract disease (pneumonia) with a low attack rate but a relatively high mortality rate, such as that which occurred among Pennsylvania legionnaires in Philadelphia in 1976 (see the Extent and Significance of the Problem Section in Chapter II). Unless otherwise specified, the term "legionellosis" as used in this document refers only to legionnaires' disease.

Sources of Infection

Soil. Although anecdotal evidence of Legionella infection carried by windblown soil or dust particles exists, there are no reliable experimental data to document this (Thacker et al., 1978; Marks et al., 1979; Conwill et al., 1982; Storch et al., 1979b). Also, legionellae have been isolated from soil only rarely and always in juxtaposition to surface water containing the bacteria (Politi et al., 1979; Cordes et al., 1980a). Organisms morphologically resembling legionellae and staining with fluorescein-labeled antibodies for L. pneumophila were seen in large numbers in some soil samples and in some soil animals, but they were not isolated (Cherry et al., 1978). A high research priority should be the reexamination of soils using new techniques designed to permit the isolation of legionellae from mixed flora (see Chapter IV). These include serologic screening with monoclonal antibodies, heat and acid treatment of the samples, and plating on selective media.

Water. Legionellae have rarely been isolated from wastewater (Cherry et al., 1982; Bercovier et al., 1984). Their natural habitat appears to be fresh surface waters such as lakes and streams, especially thermally-polluted waters (Fliermans et al., 1981a). They would probably be found in almost any site examined if the efficient methods recently developed were used to search for them (see Chapter IV). They may be present in estuaries fed by fresh waters but they have not been found in the open seas (Fliermans, 1983), perhaps because of their sensitivity to sodium ions (Barbaree et al., 1983). No reports of the isolation of legionellae from groundwater have appeared. Unless groundwater samples were taken under conditions strictly controlled to prevent surface water contamination of the equipment and the source, the interpretation of positive results would be equivocal. The presence of legionellae in potable water is well documented (see the Internal Plumbing Systems Section in Chapter III). It has been suggested, but not proven by isolation of cultures of legionellae, that rainfall may contain these bacteria (Turner et al., 1984). Basically, legionellae have the characteristics of thermoduric organisms, although they have been isolated from ice-covered rivers and exist in high concentrations in the cold waters of some lakes (Fliermans, 1983; Fliermans, 1980). Legionellae were frequently isolated from thermally altered waters as well as from natural thermal springs at temperatures $\geq$45°C (Fliermans et al., 1981a; Fliermans, 1983). The highest temperature of a natural thermal habitat from which these organisms has been isolated is 63°C (Fliermans et al., 1981a). Some natural thermal habitats have densities of legionellae that exceed those in natural nonthermal habitats by 10^2-10^3 (Fliermans, 1983). Thus, multiple opportunities exist for human and animal contact with legionella.

What happens when the legionellae "come in out of the cold" and position themselves to be a threat to human health? Other than two isolated anecdotal incidents, there is no evidence that humans get legionellosis by direct contact with ambient waters through recreational activities. The role of cooling towers and other heat-rejection systems in serving as a source of legionellae for human infection was discussed in the Nebulizers, Humidifiers and Whirlpools Section in Chapter III. In a few outbreaks substantial evidence exists to implicate these devices; in the majority of outbreaks the evidence is incomplete or questionable.

Pathophysiologic Characteristics of Legionellae

The pathophysiologic nature of legionellae is essential to an understanding of their relationship to disease production in animals and in humans. Quantitative data on infectivity of legionellae for humans has not been reported. Some animal data may be extrapolated to humans but not without serious qualifications. There appears to be a consensus in the scientific community that infection of humans by legionellae usually occurs by the respiratory route as a result of inhalation of aerosols containing the bacteria. Berendt et al. (1980) determined that the median 50% infectious dose of the L. pneumophila Philadelphia 1 strain for male Hartley guinea pigs by the aerosol route was <129 bacteria. The 50% lethal dose was 1.4×10^5 organisms, or ~1000 times greater than the 50% infectious dose. Severity of fever and serologic responses were dose related. Weight loss, fever and seroconversion were judged to be the only dependable clinical criteria of aerosol infection.

Huebner et al. (1984) exposed guinea pigs to a series of inhaled and retained doses ranging between 10 and 5000 CFU per animal of a Philadelphia and a Pontiac strain of L. pneumophila. Neither the strain number, the method of preparing the inoculum, nor the exposure apparatus was given. As shown in Table VI-2, the lowest retained infectious dose (fever production) was 10 CFU of the Pontiac isolate and 20 CFU of the Philadelphia isolate. These are the doses that induce fever in 50% of the animals (FID_{50}). The LD_{50} of the animals was in the range of 500-5000 retained CFU (see Table VI-2). There was no significant difference in the infectivity of the Philadelphia and Pontiac strains for guinea pigs; in humans, however, the former produced an acute pneumonia whereas the latter produced a mild, febrile disease (Pontiac fever). The guinea pig model supplied no evidence for a disease syndrome analogous to Pontiac fever in humans.

Baskerville et al. (1983c) were unable to explain why guinea pigs challenged with L. pneumophila developed serum antibodies after each infection but were not protected against a lethal aerosol challenge. The strains used were L. pneumophila serogroup 1 (74/81) and serogroup 3 (166/81) isolated from water and subcultured only three times before use. Fitzgeorge et al. (1983) used the same strains and, in addition, a serogroup 1 strain (NCTC 11192). The latter strain was nonpathogenic to guinea pigs by the respiratory route in larger doses ($2x10^6$) than the doses ($4x10^5$ and $3x10^5$) of the other two strains that killed 100% of the challenged animals. By the i.p. route, however, all three cultures appeared equally virulent.

In the same publication (Fitzgeorge et al., 1983), rhesus monkeys were challenged by aerosol with the serogroup 1 (74/81) strain at doses ranging from $1x10^6$-$6x10^6$ viable cells. The largest dose produced fever in 7/8 monkeys and an antibody response in 5/8. None of the monkeys died of the infection, but 4/7 had L. pneumophila in the lungs by culture at autopsy. Calculations based on weight and lung surface area showed that the highest dose of organisms ($\sim 10^6$) given the monkeys was ~30-fold less than the $\sim 10^4$ cells that produced lethality in guinea pigs.

Baskerville et al. (1983a) exposed guinea pigs for 5 minutes and rhesus monkeys for 10 minutes to an aerosol of the serogroup 1 (74/81) strain of L. pneumophila (see Table VI-2). Estimates of the respiratory minute volume indicated that 50% of the inhaled bacteria was retained in the lungs. On this basis, three groups of guinea pigs received $2.4x10^3$, $3.7x10^4$ and $4.9x10^5$ viable bacteria, respectively. The monkeys received only a single dose of $6x10^6$ viable bacteria. All guinea pigs were pyrexic, and all those receiving the two largest doses were dead on the third day (see Table VI-2). In the guinea pigs 11/12 receiving the $2.4x10^3$ dose survived;

those receiving the 2×10^2 dose had no symptoms. Temperature increases of 0.5-1.8°C were seen in 4/6 monkeys; two did not develop fever (see Table VI-2). The dose received by the monkeys was calculated to be comparable to that of the guinea pigs, i.e., a 10-fold higher retained dose for lung volume ~12-fold greater. The alveolar surface area of the monkeys was, however, calculated to be 32 times larger than that of the guinea pigs, so a 10-fold greater retained aerosol dose for the monkeys represented a relatively lower infecting dose.

Katz and Matus (1984) attempted to infect three groups of guinea pigs with a serogroup 1 strain of L. pneumophila by 1) drinking water, 2) gastric intubation, and 3) i.p. injection, respectively. The range of doses for group 1 was 10^3-10^8 bacteria per animal; for group 2 it was 10^3-10^{10} bacteria; and for group 3 it was 10^6 bacteria. A total of 52% of the guinea pigs in group 1, 63% in group 2, and 83% in group 3 had fever compared with 2.2% of the controls. Fever lasted for ≤ 3 days in groups 1 and 2, but the animals had mild pneumonitis and splenitis. In group 3, fever lasted longer and 63% of the guinea pigs died. L. pneumophila was isolated from the lungs and spleen of 5/47 animals in groups 1 and 2. Thus the data suggested that <1000 ingested L. pneumophila organisms produced fever in guinea pigs. There were difficulties in interpreting this study. The investigators could not be certain that aerosols were not produced and inhaled during drinking or while removing the intragastric delivery tube, resulting in the observed effect. The investigators, however, did recognize the problem and took precautions during the study. Another difficulty, possibly related to the aerosol question, is that no dose-response pattern was observed between 10^3 and 10^8 organisms.

Hambleton et al. (1982) observed that, unlike i.p. infection with legionellae, guinea pigs and other animals infected by the aerosol route seldom showed extrapulmonary symptoms such as diarrhea, kidney or liver failure, or neurologic disturbances. Possibly the frequency of diarrhea and neurologic disturbances in human cases may be the result of infection by another route, perhaps ingestion.

In ingestion experiments with guinea pigs reported by Conner and Gilbert (1979), the animals imbibed an estimated total dose of 2.3×10^{8}-2.3×10^{9} cells of *L. pneumophila* in their drinking water over a period of 17 days. None of the animals was clinically-ill and none seroconverted. Only three animals were used in the experiment, and the viability and virulence of the strains in their drinking water was uncertain.

Baskerville (1984) calculated a retained LD_{50} aerosol dose of $10^{4.5}$ and $10^{2.5}$, respectively, for the 74/81 and Corby strains of *L. pneumophila* serogroup 1 in guinea pigs. The *L. pneumophila* serogroup 3 strain (166/81) gave an LD_{50} of 10^{4} under the same conditions.

The Role of Water in Producing Infection

Water Systems. The major concern in this document is the role of drinking water in producing legionellosis. Approximately 180-200 million people in the United States depend on community water supplies, and for 68.2% of these (122-136 million) the source is surface water (Cotruvo, 1983). The remainder of the population is served by noncommunity public water systems, 96.4% of which use groundwater as a source. Thus, noncommunity water systems is not discussed here because they serve only 10-20% of the total U.S. population and no published data are available on groundwater as a habitat for legionellae.

Sporadic Legionellosis. The 122-136 million people who are supplied surface water by community public water systems are potentially subject to infection by legionellae from all sources known and unknown. Excluding the 894 cases resulting from the 19 outbreaks (see Table III-5; England, 1981), the sporadic case rate for Legionella pneumonia in the United States was calculated to be 12/100,000, or ~25,000 cases per year. This figure was based on a serological survey and may be understated (Foy et al., 1979; Fraser, 1980). Patients with serious sporadic Legionella infections are admitted to hospitals where, in some instances, there may be confusion about the source of the infection, i.e., community acquired or nosocomial. The question of source may be complicated further if the hospital is experiencing concurrent nosocomial cases of LD.

A salient fact pointed out by Muder et al. (1983) is that in many institutions the true incidence of LD as a cause of nosocomial pneumonia is unknown. This is also the case for sporadic legionellosis. In a hospital where LD had never before been documented, four cases of nosocomial and three cases of sporadic community-acquired LD were diagnosed in one 2-month period (Muder et al., 1983). These seven cases represented 14.3% of all the nosocomial pneumonias and LD was the third most common cause of these infections. During the same period, 64% of all sampled sites in the water distribution system within the hospital yielded L. pneumophila.

Nosocomial Legionnaires' Disease. Nosocomial pneumonia is defined as a lower respiratory tract infection that develops during a patient's hospitalization, is neither present nor incubating at the time of admission, and usually is not manifest in the first 47-72 hours after admission (Simmons and Wong, 1982). When the term "sporadic nosocomial legionellosis" is used, it refers to sporadic rather than epidemic infection within the hospital.

In 1981 there were 6933 registered hospitals in the United States (Hospital Statistics, 1982). Excluding psychiatric and all other special hospitals, there were 6069 general hospitals and hospital units of institutions. These hospitals had 38,036,015 admissions and 301,972,692 inpatient days with a daily census of 827,656 patients. These patients, as a group, would appear to be those in the U.S. population at greatest risk of contracting legionellosis.

What are the reasons for this? Of the 19 outbreaks (see Table III-5), 7 occurred in hospitals; 1 involved four different hospitals. Of 894 cases in the 19 outbreaks, 343 occurred in hospitals although cases were occurring simultaneously in the community in at least one of the hospital outbreaks (see Table III-5).

The existence of nosocomial legionellosis is well established. Temporally, it differs from non-nosocomial sporadic and epidemic legionellosis in that cases may be more likely to have onset throughout the year rather than being concentrated in the summer and fall months (Cohen et al., 1979). Some reports of nosocomial legionellosis are listed in Table IX-1. The great

TABLE IX-1

Reports of Nosocomial Legionellosis

Location	Years	Deaths/Cases	Reference
Detroit, MI	1978	1/8 (12.5%)	Saravolatz et al., 1979a
Pittsburgh, PA	1979-1980	12/34 (18%)	Brown et al., 1980
Pittsburgh, PA	1979-1980	NR/32	Yu et al., 1982
Pittsburgh, PA	1981	NR/19	Muder et al., 1983
Pittsburgh, PA	1979	5/8 (62.5%)[a]	Myerowitz et al., 1979
Iowa	1981	11/24 (46%)	Helms et al., 1983
CDC Report	NR-1979	10/263 (3.8%)[b]	Cohen et al., 1979
Los Angeles, CA	1977-1978	15/49 (30.6%)	Haley et al., 1979
Los Angeles, CA	1977-1978	16/64 (25%)[c]	Kirby et al., 1980
CDC Report	NR-1980	22/71 (31%)	England and Fraser, 1981

[a] All eight patients were immunosuppressed and infected by _L_. _micdadei_ (PPA).

[b] Autopsy specimens from 40 hospitals examined by stains.

[c] Probably includes the 49 cases of Haley et al. (1979).

NR = Not reported

variation in death rates is evident and partially explained by 1) late diagnosis, 2) use of inappropriate therapy, and 3) diverse medical conditions of patients at the time of infection. The combined mortality rate for the cases in the eight reports for which complete data are given (see Table IX-1) is 92 of 521, or 17.7%.

Cohen et al. (1979) estimated that sporadic nosocomial legionellosis accounted for 3.8% of the fatal cases of nosocomial pneumonia. England and Fraser (1981) stated that 73 (5%) of 1432 cases of sporadic LD were of nosocomial origin. In Pittsburgh, PA, 29% of all cases of nosocomial pneumonia were caused by *L. pneumophila* (Yu et al., 1982). *L. pneumophila* was also the most common cause of pneumonia (22.5%) produced by a single pathogen (Yu et al., 1982). A prospective study of pneumonia in another hospital never having had a documented case of LD revealed that 14.3% of all nosocomial pneumonias were caused by *Legionella* (Muder et al., 1983). In a third Pittsburgh hospital (Veterans Administration), 15 of 32 (47%) of the cases of nosocomial pneumonia were caused by legionellae (Muder et al., 1983). Storch et al. (1979b) found 7% of their first 100 sporadic cases to represent possible nosocomial infection. A summation of these six reports gives a total of 131 cases of sporadic nosocomial LD among 1929 patients with nosocomial pneumonia for an incidence of 6.8%.

The combined infection rate for total nosocomial pneumonias reported in 1980 by the hospitals that participated in the National Nosocomial Infection Study (NNIS) was 5/1000 admissions (Simmons and Wong, 1982). Applying this rate to the total number of admissions (38 million, rounded) to registered hospitals in 1981 provides a figure of 190,000 expected annual cases of nosocomial pneumonia. If a conservative figure of 5% (see previous paragraph) is used to represent the incidence of sporadic and epidemic nosocomial LD pneumonia among all cases of nosocomial pneumonias, the annual number of cases can be calculated (Table IX-2). These 9500 cases constitute 38% of the 25,000 cases of LD estimated to occur in the United States annually (Fraser, 1980). The difference, 15,500 cases, represents an estimate of all other LD cases (sporadic, endemic or epidemic) that occur annually. It is emphasized that these estimates are, at best, only a rough guide; at worst they may represent seriously flawed assumptions. Data appropriate for better estimates have not been found.

Sources of Nosocomial Infection -- The potential sources of nosocomial infection are discussed in the Specific Occurrence Section in Chapter III and are listed in Table VII-7. The potable water sources of potential importance for nosocomial legionellosis are showers, faucets (taps), hot water tanks, storage tanks, respiratory therapy equipment and humidifiers. Because of the preference of legionellae for warm aqueous environments, warm water sources should be given special attention.

In the Philadelphia outbreak of 1976, ill delegates were significantly ($p=0.01$) more likely to have drunk water in the outbreak hotel than were well delegates (Fraser et al., 1977). It has been shown that *L. pneumophila* cannot only remain viable in tapwater for a year or more but the bacteria actually grow in tapwater at temperatures between 32 and 42°C (Skaliy and McEachern, 1979; Yee and Wadowsky, 1982). Yu et al. (1981) determined that a *Legionella* culture colonized the nasopharynx of a volunteer for at least 0.5 hour. From this they postulated that patients drinking contaminated

TABLE IX-2

Prevalence of Nosocomial Legionnaires' Disease in the United States

Total admissions, registered hospitals (1981) (round numbers)	38,000,000[a]
Total nosocomial pneumonia cases at rate of 5/1000 admissions	190,000[b]
Incidence of LD in cases of nosocomial pneumonia	5%[c]
No. of cases of nosocomial LD pneumonia	9,500
No. of cases of LD pneumonia (total)	25,000[d]
Proportion of all cases of LD that are nosocomial pneumonia	38%
No. of sporadic cases of LD pneumonia (by difference)	15,500 (62%)

[a] Hospital Statistics, 1982

[b] Simmons and Wong, 1982

[c] 5% is a key figure representing an assumption that may be seriously biased (see text).

[d] Fraser, 1980

water may be colonized with legionellae that may be aspirated into the lungs to produce infection. Saravolatz et al. (1984) found that the oropharynx of 5.7% of their patients was colonized with legionellae, in one patient for a long period of time.

Guinea pigs were infected with aerosols of hot (48°C) tapwater containing a strain of L. pneumophila that was epidemiologically associated with six cases of nosocomial LD (Meenhorst et al., 1983).

A patient was presumably infected by gastric lavage with tapwater found to be contaminated with L. pneumophila (Dournon et al., 1982). A community-acquired case of L. micdadei infection in Sweden was thought to be related to drinking rainwater from a barrel (Back et al., 1983).

An unusual serogroup of L. pneumophila in hospital shower water was presumed to have caused infection in three patients who became ill 2-10 days after showering and from whom the same organisms were isolated (Cordes et al., 1981).

Numerous reports exist of the contamination of potable water with legionellae in hospitals and other patient-care institutions. In many cases these organisms have been associated with epidemics or with sporadic cases of LD. In other cases, disease has not been associated with the presence of legionellae in potable water, a not unexpected finding for an opportunistic pathogen (Desplaces et al., 1984).

The Potable Water Section in Chapter III of this criteria document should be consulted for other examples of the close association of human infection with drinking water. The Hospitals Section in Chapter III contains a detailed discussion of the association of the internal plumbing of hospitals with the presence of nosocomial legionellosis in those institutions.

Risk Factors -- The genus *Legionella* has the characteristics of an opportunistic pathogen (Myerowitz et al., 1979; Taylor et al., 1981). Persons at high risk are those with underlying diseases, especially cancer (immunosuppression), renal failure (dialysis), and those receiving immunosuppressive drugs. Relative risk data given in Tables IX-3 and IX-4 were calculated retrospectively for the various risk factors after determining that the data were statistically significant by chi-square or other appropriate tests. The relative risk is given by $R = \frac{ad}{bc}$ where, for example, a represents the number of smokers with LD, d represents nonsmokers without disease, b represents smokers without disease, and c represents nonsmokers with disease. Various risk factors and their relative risks are shown in Tables IX-3, IX-4 and IX-5 for a number of epidemics and sporadic case studies. Smoking is an important risk factor that has been quantitated in some studies (see Table IX-3). Both age and sex are clearly risk factors, the latter being partially occupationally related. Abuse of alcohol is another risk factor.

Saravolatz et al. (1984) found *Legionella* to be the etiologic agent of 16/40 (40%) of their cases of pneumonia in renal transplant patients. During the 6 years in which the cases occurred, no patient was infected during the period January-March, whereas most non-*Legionella* pneumonia

TABLE IX-3

Risk Factors for Legionellosis*

	Relative Risk	Reference
Smoking		
1976 Philadelphia outbreak	3.4	Fraser et al., 1977
Sporadic case control study	1.6(<1/2 PPD) 4.2(>1 PPD)	Storch et al., 1979b
Vermont outbreak	1.7	Broome et al., 1979
Norwalk outbreak	1.2-1.5	Wong et al., 1979c
Age		
1976 Philadelphia outbreak	0.5(<40)-1.9(≥70)	Fraser et al., 1977
Sex (M vs F)		
1976 Philadelphia outbreak	2.8	Fraser et al., 1977
Vermont outbreak	2.4	Broome et al., 1979
Norwalk outbreak	3.7	Wong et al., 1979c
Alcohol		
Sporadic case control study	3.5-4.8 (>3 drinks/day)	Storch et al., 1979b
Norwalk outbreak	5.3	Wong et al., 1979c
Immunosuppressive agents		
Fatal nosocomial LD study (steroids)	2.5	Cohen et al., 1979
Los Angeles outbreak	9	Haley et al., 1979
Underlying disease		
Sporadic case control study	2.0	Storch et al., 1979b
Vermont outbreak (cancer or dialysis)	1.8	Broome et al., 1979
Norwalk outbreak (renal failure, cancer, immunosuppression)	6.0	Wong et al., 1979c

*Source: Adapted from England, 1981

PPD = Packs per day

TABLE IX-4

Prevalence of and Relative Risk Associated with Selected Underlying Conditions or Treatment for 83 Persons with Sporadic Nosocomial Legionellosis, United States, October 1974-April 1980[a]

Conditions or Treatment	Observed Cases (%)	Expected Cases[b]	Relative Risk
Renal dialysis or renal homograft	11 (15.7)	0.03	340
Immunosuppressive medication	28 (54.9)	4.8[c]	26[c]
Cancer[d]	17 (26.2)	1.6	11
Chronic bronchitis or emphysema	12 (19.7)	3.2	3.7
Smoking[e]	29 (46.0)	15.5	1.9
Diabetes mellitus	4 (6.5)	3.0	1.3

[a] Source: England and Fraser, 1981

[b] Adjusted for age and sex (and race for renal dialysis or homograft patients).

[c] Mantel-Haenszel

[d] History of cancer (10 selected sites or types)

[e] ≥10 cigarettes/day

TABLE IX-5

Other Risk Factors for Legionnaries' Disease

Risk Factors	Reference
Bone marrow transplants	Kugler et al., 1983
Administration of corticosteroids and other immunosuppressive agents	Gump et al., 1979; Myerowitz et al., 1979
Renal transplantation	Bock et al., 1978; Rogers et al., 1979; Dowling et al., 1984; Taylor et al., 1981
Race (white vs. black)	Helms et al., 1981
Related to associated respiratory infection, travel, and construction	Storch et al., 1979b
Cardiac, pulmonary, and renal disease; diabetes mellitus; and peptic ulcer	Kirby et al., 1980
Chronic underlying diseases; immunocompromising conditions	Beaty et al., 1978
Home air-conditioners	Broome et al., 1979

occurred during this period. In a second study of 70 renal transplant patients, oral colonization with Legionella occurred in four patients (5.7%) and in one patient who harbored two different Legionella species for 6 months without infection. Of 241 reported cases of LD, 63% had significant underlying disease (Meyer and Finegold, 1980).

Contacts of patients with LD, laboratory staff who process patients' specimens, and air-conditioning maintenance personnel have been assumed to be at risk of infection by Legionella, but there is very little evidence to support this. Saravolatz et al. (1979b) found a significant difference ($p<0.02$) in the prevalence of antibody titers (hemagglutination) in hospital employees in contact with patients or their specimens and employees who had no known exposure to infected patients or to the organism. During the Philadelphia outbreak in 1976, however, there was no evidence of secondary cases among contacts of patients with pneumonia (Fraser et al., 1977) or of infection among personnel handling specimens associated with the epidemic (McDade et al., 1977).

Yu et al. (1983) could find no evidence for person-to-person transmission of L. pneumophila among 47 contacts of 17 patients with proven LD. Using the ELISA, no contacts of patients seroconverted. The authors concluded that contacts of patients should be reassured about the lack of contagiousness of LD. They also suggested that routine isolation measures could be discontinued and selected cases managed as outpatients.

A possible infection of a pathologist who performed an autopsy on a victim of LD was reported by Van den Bergen et al. (1979). A possible person-to-person spread of L. pneumophila also was reported by Campbell-Love et al. (1978).

High-risk air-conditioning maintenance personnel were surveyed for antibody titers for L. pneumophila serogroup 1 (Goldman and Marr, 1980). Their titers were compared with a similar group of employees from the same company who did not have contact with cooling towers or with air-conditioners. The prevalence of titers (≥128) for the high-risk workers was 19% compared with 22.2% for the low-risk group. There was no significant difference in the history of pneumonia between these groups.

Percentage of Cases Due to Drinking Water Ingestion or Inhalation -- It is not possible, at this time, to calculate what percentages of cases of Legionella infection are due to either inhalation or ingestion of drinking water. The data most relevant to this topic are found in the Potable Water Section in Chapter III for drinking water in general and the Internal Plumbing Systems Section in Chapter III for drinking water related to hospitals, hotels, clubs, public buildings, homes and factories.

Person-to-person transmission of Legionella infection has rarely been suspected and never proven. Fecal-oral transmission has not been reported. Foodborne outbreaks caused by legionellae are unknown.

Summary

The natural habitat of legionellae appears to be surface water. Because legionellosis (including both LD and Pontiac fever) has been described in at least 26 countries, it is reasonable to assume that surface waters serve as a global habitat for legionellae. They have been found only in fresh or brackish waters; their presence in groundwater has not been documented.

Potable waters containing legionellae constitute a health hazard primarily for high risk populations. It is assumed that small numbers of legionellae present in surface waters escape water treatment procedures. They then pass into the distribution system, eventually finding niches suitable for maintaining viability and growth in components of terminal plumbing systems. Legionellae were shown to survive low levels of chlorine for rather long periods of time, being much more resistant than *Escherichia coli*. Isolation of legionellae from water in the extramural portions of distribution systems has not, however, been reported. The bacteria have a predilection for warm water and, therefore, are more likely to be found in larger concentrations in components of hot water systems at temperatures up to ~55°C than in cold water, especially at temperatures below 20°C.

Cooling towers have been epidemiologically associated with several severe outbreaks of LD. Legionellae may gain entrance to cooling towers and whirlpools from either potable or untreated water sources. Open air-conditioning systems and whirlpools may also be contaminated by airborne legionellae, but this has not been proven.

Although it is often difficult to separate outbreaks of LD from sporadic cases occurring in hyperendemic areas, more than 30 epidemics of LD have been reported worldwide, over 20 of them being in the United States. Cases included in a listing of the first 19 outbreaks in the United States totaled 894.

It has been estimated that 25,000 cases of LD pneumonia occur in the United States each year. If 5% (a conservative figure) of the 190,000 cases of nosocomial pneumonia estimated to occur in the United States each year are caused by LD, the expected number of cases of nosocomial LD pneumonia will be 9500. Then, by difference, 15,500 (62%) of all LD pneumonia cases are sporadic. Although LD is a reportable disease in the United States, probably only a small fraction of cases are reported to the Centers for Disease Control.

A case of LD is defined as an illness characterized by cough and fever (temperature $\geq$38.9°C) or any fever and chest X-ray evidence of pneumonia. Chills, dyspnea, headache, chest pain, myalgias, malaise, diarrhea and mental confusion also are common symptoms. Pontiac fever is an acute but mild febrile illness without significant upper or lower respiratory tract disease. Only ~500 cases have been reported.

Mortality in LD epidemics has ranged from 8-67% but is usually $\leq$25%. In 2470 reported cases of nosocomial LD occurring in the period 1970-1982, the mortality rate was 20%. The case fatality rate for patients with nosocomially acquired LD who did not receive erythromycin was reported in one large study to be 55%; for patients receiving the drug the mortality rate was 13%.

Quantitative data on infectivity of *L. pneumophila* for humans has not been reported. Data derived from studies with guinea pigs indicated that retained aerosol doses as low as 10 CFU constituted an LD_{50}. Doses producing LD_{50} effects ranged from ~10^3-10^5 CFU depending upon the strain of *L. pneumophila* and a variety of other factors.

The most important risk factors for LD are serious underlying diseases such as cancer (immunosuppression), renal failure (dialysis and transplantation), therapy with immunosuppressive drugs, diabetes, chronic bronchitis and emphysema. Age and sex are also predisposing factors.

With the caveat that the mechanisms for transmission of legionellae from the environment to the human lungs are unproved, the most acceptable hypothesis is that of inhalation or ingestion of contaminated water. Evidence for aerosol transmission comes from studies of several outbreaks in which Legionella contaminated cooling towers or evaporative condensers were strongly associated epidemiologically with LD. Evidence for infection produced by transmission of legionellae in aerosols of potable water is very strong but lacks rigorous scientific proof. Legionnaires' disease associated epidemiologically with the presence of legionellae in potable water from showers, faucets, hot water tanks and respiratory therapy equipment is a serious threat to susceptible people.

Although guinea pigs have been infected by ingestion of legionellae, there is no convincing evidence that humans are naturally infected in this way. Likewise, person-to-person transmission has not been convincingly demonstrated. Infection by means of food or other vehicles has not been reported.

The hypothesized chain of infection with LDB begins with the presence of the bacteria in untreated surface water. It proceeds through the water treatment process, which fails to remove the legionellae, through the distribution system, and finally results in deposition of the organisms in warm water sites of the plumbing system. Here the legionellae are sequestered in niches favorable to their survival and growth, such as the sediment of hot water tanks and the internal fittings of showerheads and faucets that are colonized by the bacteria. From these concentrators and amplifiers, aerosols are dispersed containing legionellae that are infectious to high-risk persons under certain poorly defined conditions.

The two most successful methods for controlling LDB in potable water are chlorination and heating. Hyperchlorination to cleanse the plumbing system followed by maintenance of free chlorine residuals in the range of 1-2 ppm is usually effective. Raising the hot water temperature to ~70°C temporarily to kill the LDB and then holding the water at 55°-60°C has been effective. A combination of these two methods used intermittently or continuously may be useful. There seems to be no justification for treating water at treatment plants to remove legionellae unless it can be shown experimentally to be cost effective. Many opportunities exist for introduction of LDB into the water during storage and in the distribution system by means of broken pipes, back siphonage, cross connections, etc.

Although patient-care institutions want to prevent nosocomial infections with LDB, there are no agreed-upon guidelines for control. Legionellae are common in potable water but relatively few cases of legionellosis occur. Generally, control measures have not been considered cost effective unless epidemic or frequent sporadic nosocomial cases are occurring. The problem is that the mechanisms of transmission of, and infection by, legionellae are still too poorly understood to be amenable to specific recommendations for control. Information is needed on 1) the possible undisclosed sources of legionellae, 2) nutritional factors influencing their survival and growth in potable water systems, 3) size and concentration of aerosol droplets dispersed by potential disseminators, 4) factors affecting virulence, and 5) ingestion versus inhalation as a route of infection.

X. CONTROL OF LEGIONELLA

Treatment Chain

Control of Legionellae in Community Water Systems Before Entering the Distribution Network. With the caveat that many aspects of the transmission mechanism of legionellae from their environmental habitats to the human lungs are unproven, the most acceptable hypothesis is that of inhalation or ingestion of contaminated water. The widespread distribution of these bacteria in nature, the evidence derived from many serologic surveys, and the worldwide occurrence of infections argue that people are intermittently, at least, transient hosts for legionellae because of our intimate relationship with water. Because there are only two reported cases of legionellosis that might have resulted from immersion in surface waters, this is not considered an important means of infection. Ancillary information possibly bearing on the normally high resistance of humans to infection comes from the experience of a group of workers involved in processing large volumes of surface water in a field laboratory over a period of several years. During this time neither disease nor seroconversion occurred among the group, although almost all of the water samples had high DFA counts for legionellae and these bacteria were isolated from guinea pigs infected with many of the water samples.

Legionella infection attributed to heat-rejection equipment such as cooling towers is not emphasized in this section of the document; instead, consideration is given to the control of *Legionella* in the drinking water of community public water supplies whose source may be either surface water or groundwater. There is some indication that *Legionella* may be minimally present or absent from some groundwaters (Fliermans et al., 1982; Spino et al., 1984). In small systems using groundwater, disinfection may be the only treatment needed before distribution of the water (Engelbrecht, 1983). Surface waters may be stored and then subjected to coagulation-flocculation, settling, filtration, and disinfection. In some systems, treated and disinfected waters may be stored in open reservoirs for a period of time before being distributed. Thus, the opportunity exists for recontamination of the water with bacteria, algae, and protozoa, and for regrowth of pathogens.

Studies on prechlorination of water supplies have shown that the main advantage of the procedure was improvement of the effectiveness of the rapid sand filtration step although coliform densities were also usually significantly reduced (Engelbrecht, 1983). Most water treatment plants in the United States use chlorine for posttreatment or terminal disinfection. Although extrapolation of laboratory studies to treatment plant situations is somewhat hazardous, Kuchta et al. (1983) reported that both L. pneumophila and L. micdadei were much more resistant to chlorine than was E. coli. At 21°C, pH 7.6, and 0.1 mg/ℓ of free chlorine residual, a 99% kill was achieved in less than 1 minute for E. coli compared with 40 minutes for L. pneumophila. The contact time for a 99% kill of L. pneumophila at 4°C was twice as long as it was at 21°C. The authors concluded that legionellae can survive low levels of chlorine for rather long periods of time.

Tison and Seidler (1983) examined raw water (no chlorine residual) and three kinds of distribution water supplies: 1) those treated by chlorine (free residual 0.2-0.6 ppm); 2) those treated by sand filtration and chlorination (free residual 0.0-0.4 ppm); and 3) those treated by flocculation, mixed-media filtration, and chlorination (free residual 0.5-2.0 ppm).

Legionella were enumerated by DFA tests and all distribution waters contained about 1 $\log_{10}$ fewer LDB-like cells than did the raw waters, i.e., 10^3-10^4 per liter. The significance of these results is questionable because the authors were unable to culture any legionellae.

A comparison of agar-passaged and tapwater-grown strains of *L. pneumophila* with respect to chlorine resistance showed that the latter were considerably more resistant (Kuchta et al., 1984). The conditions of the test were 0.25 mg/ℓ free residual chlorine, 21°C, and pH 7.6-8.0. The authors had shown previously that *L. pneumophila* cultures maintained on agar media were much more resistant to chlorine than were coliforms (Kuchta et al., 1983).

Control of Legionellae in the Distribution Network.

General Considerations -- Legionellae are found in raw waters and at the endpoint of the distribution system, but the history of these organisms as they pass into and through the water treatment plants is unknown. Occurrences within the distribution system before arrival of the water supply at the user's storage tank, faucet, shower, etc., also are unknown. The organisms may survive the treatment and disinfection process and pass intact through the distribution system. In addition, opportunities exist for their introduction into the system by means of broken or corroded piping, repair of existing mains, installation of new mains, back siphonage, and cross connections, any of which may contaminate the water supply. In older distribution systems, especially those dependent on gravity flow, deterioration of piping may be so severe that the treated water comes in intimate contact with soil and is subject to infiltration by surface water. Thus, legionellae may be introduced into potable water by these routes.

Legionellae surviving initial water treatment may colonize pipe joints and corroded areas or adhere to the surface or sediment of storage tanks, especially those constructed of wood. Here, they may find a habitat suitable for survival and regrowth (Engelbrecht, 1983). Cul-de-sacs, intermittently used storage tanks, and other sites in which waterflow is absent or restricted may also be appropriate habitats for legionellae.

New distribution systems or components thereof that were not appropriately cleaned and disinfected before being put into use may introduce legionellae and other organisms into the system. It may not be coincidence that some of the serious outbreaks of legionnaires' disease occurred in newly opened institutions or buildings. Wadsworth Medical Center in Los Angeles was first occupied in March 1977; in May 1977 the first case of LD was identified (Haley et al., 1979). In Columbus, OH, in June 1977, construction was started on a new hospital wing; in August a cluster of cases of LD was identified in the adjacent building (Marks et al., 1979). A second hospital in the area, also undergoing construction of a new wing, experienced cases of LD. Infection was hypothesized to be related to air-conditioning and to excavations. In retrospect, one can speculate that construction activities may have included intervention into the water supply mains with introduction of contaminated water or, possibly, disturbance of sediment and sloughing of scale bearing high concentrations of LDB by means of hydraulic shock or other perturbations.

On March 5, 1981, 21/24 cases of LD occurred in patients admitted to a newly opened addition of the University of Iowa Hospital (Helms et al., 1983). Sixteen of these were associated with one hospital unit and the first case appeared 11 days after the unit was opened. Again, attention is directed to the possibility of unsterilized components of a new distribution system or at least to stagnant water that provided conditions for increased growth of legionellae. L. pneumophila was isolated from faucets, showers, and a hot water tank.

Although a San Francisco outbreak was seemingly associated with a cooling tower and potable water was not found to be a risk factor, it is interesting that the 14 cases occurred in a building only 6 months old (Conwill et al., 1982). Furthermore, the last three floors of the building were first occupied only a few days before the first two cases occurred on March 3, 1980. Twelve additional cases had onset between March 6 and March 15. An excavation was in progress at a site across the street from the building, and the outbreak was preceded by very heavy rainfall in February, i.e., 7.62 inches. An analogous situation of excavation and heavy rainfall was recorded during the outbreak at St. Elizabeth's Hospital in 1965 (Thacker et al., 1978).

Hambleton et al. (1983) showed convincingly that aerosols of L. pneumophila remained viable for as long as 2 hours at a relative humidity of 65%. Also, stationary-phase, broth-grown organisms were much more stable in aerosols than exponential-phase organisms. One would expect that a slow-growing organism like L. pneumophila emanating from potable water amplifiers would have low metabolic activity and good aerosol stability at a favorable relative humidity.

Muder et al. (1983) made a cogent argument for all hospitals to make intensive and parallel searches for undiagnosed cases of nosocomial LD and in-house water sites contaminated with legionellae. This was based on a study in a hospital where LD had never been documented, but which found upon examination that 14.3% of the nosocomial pneumonias were caused by legionellae and that 64% of tested sites in the water system were positive for LDB.

Finally, fountains, waterfalls, and pools within enclosed malls may be a hazard because the water is frequently vigorously agitated with ensuing aerosol production. L. pneumophila has been isolated from such pools (Cherry and Orrison, 1979).

Observations such as those cited in this chapter confound rational interpretation of the role of various mechanisms in the transmission of legionellosis.

Use of Chlorine and Heat -- Engelbrecht (1983) listed the requirements for a microorganism to colonize a water distribution system as follows: it must 1) enter the system, 2) become associated with a suitable habitat, 3) be able to obtain nutrients from the water either directly or indirectly, and 4) be able to reproduce itself. Considerable evidence has accumulated to show that legionellae are sequestered in habitats within the endpoint domain of distribution systems. These have been discussed in Chapter III (see sections on Internal Plumbing Systems and Nebulizers, Humidifiers and Whirlpools) of this document and will be cited here only from the viewpoint of control. Legionellae have a predilection for warm water habitats as shown by their high concentration in thermally-altered surface waters, in heat-rejection equipment, and in their isolation from artificially-heated water systems. It was shown that L. pneumophila survived for 1 year in tap water (Skaliy and McEachern, 1979). Yee and Wadowsky (1982) demonstrated that naturally-occurring L. pneumophila never grown on artificial media could grow in tapwater at 32, 37 and 42°C. Non-Legionella bacteria grew at 25, 32 and 37°C but not at 42°C. The quantitative increase in Legionella was ~3 $\log_{10}$ at 37 and 42°C. Apparently, indigenous Legionella do not grow at 42°C in tapwater in the absence of non-Legionella bacteria (Yee, 1984). The source of the L. pneumophila was a showerhead and sediment from a hot water tank. Earlier, Wadowsky et al. (1982) isolated legionellae from faucet assemblies, showerheads, shower pipes, and from the sediment of hot water tanks in hospitals, nonhospital institutions, and from one home. Cultures were positive in hot water tanks at 30-54°C but not in those maintained at 77°C. They hypothesized that hot water tanks were a breeding site for L. pneumophila and were responsible for contamination of plumbing systems.

The design of hot water tanks is extremely important in maintaining them free of legionellae. Most residential hot water tanks are heated from the bottom near the cold water entrance pipe and are more likely to maintain a bottom temperature high enough (>55°C) to prevent growth of LDB. The water at the bottom of some of these tanks may, however, be cool enough (<55°C) to maintain viability or growth of the legionellae especially when thermostats have been kept at energy-efficient settings in conformity with recommendations of the Joint Commission on Accreditation of Hospitals (≤49°C). Also, the water as it is heated moves rapidly to the top of these tanks. Large institutional tanks are more often heated by internal steam coils or by other heaters located midway from top to bottom of the tank. The water at the bottom may not be heated sufficiently to kill legionellae. Although increasing the water temperature to ≥55°C may control LD, it requires posting of signs and extra care by hospital personnel to prevent scalding of patients.

The other effective control method is chlorination of the potable water system. It is difficult to maintain a chlorine residual in the hot water unless it is part of a closed, recirculating system. This is most satisfactorily done with an automatic, continuous chlorinator. Free chlorine residuals that are effective and maintainable are in the range of 1-2 ppm. Chlorination of hot water at these levels increases corrosion of the plumbing system and may cause maintenance problems.

Tobin et al. (1980) isolated L. pneumophila from showers in a transplantation unit experiencing cases of legionellosis. The holding tanks were superchlorinated and the shower fitting disinfected by heat at 65°C for 18 hours, but legionellae were found again 9 months later. The storage tanks, hot and cold water taps, and showerheads in a hotel experiencing four cases of legionellosis were positive for these organisms (Tobin et al., 1981b). After cleaning and chlorination (50 mg/ℓ) of the distribution system, no further cases had occurred in the ensuing 9 months.

Fisher-Hoch et al. (1981a) used hypochloride to obtain a level of 1-2 mg/ℓ of free chlorine at all cold water outlets in Kingston Hospital where legionellae were present in both cold and hot water. The free chlorine levels in the hot water could not be maintained above 0.2 mg/ℓ and legionellae were recoverable at this level. The water temperature was ~45°C, which was warm enough to drive out the chlorine and cool enough to allow growth of legionellae. The problem was solved by maintaining the water temperature at 55-60°C; no further cases have occurred.

Contaminated hot tap water (48°C) aerosolized to guinea pigs produced pneumonia caused by the same strain of L. pneumophila that was isolated from a series of patients in The Netherlands (Meenhorst et al., 1983).

Dennis et al. (1982) examined water samples from the plumbing of 52 hotels, none of which was associated with cases of legionellosis. Ten isolates of L. pneumophila were obtained from water samples from eight hotels. Seven of these were from hot water taps or hot-cold mixer showers with water temperatures ranging from 40-54°C at the time of sampling.

Attempts to eradicate legionellae from a disconnected hot water tank by filling it with water containing 50 mg/ℓ of chlorine for 24 hours followed by descaling were unsuccessful (Fisher-Hoch et al., 1982). Maintaining a constant water temperature of 70°C throughout the tank for one hour eliminated the organisms. Continued surveillance, however, revealed positive cultures for legionellae from various tap and shower fittings. An intensive study of water fittings as sources of L. pneumophila in a hospital plumbing system was conducted out by Colbourne et al. (1984a,b). In well-controlled experiments, L. pneumophila was isolated from rubber washers and gaskets but not from fiber or plastic fittings. The ability of the bacteria to multiply when in contact with the rubber fittings was demonstrated. When the rubber fittings were replaced with National Water Council approved fittings (Teflon?), L. pneumophila could not be isolated up to 1 year later. The authors concluded that shower and tap fittings that support growth of legionellae provide habitats protected from chlorine and heat. These foci may be constantly or intermittently seeded with a new inoculum of legionellae arising from hot water tanks or other amplifiers within the distribution system.

Massanari et al. (1984) controlled a nosocomial outbreak of L. pneumophila infection by shock chlorination (15 mg/ℓ) of both hot and cold water supplies. The system was then flushed and the water temperature raised to 64°C for 41 days. These measures significantly reduced the frequency of positive culture. Since the installation of a continuous-flow proportional chlorination unit that provided free chlorine levels of 8 and 7.3 mg/ℓ in hot and cold water, respectively, no new cases of legionellosis have been identified.

Baird et al. (1984) hyperchlorinated their hospital water supply at a constant level of 4 mg/ℓ of free chlorine. The rate of nosocomial LD decreased by almost two-thirds and the total numbers of LDB decreased, but the organisms persisted.

In state development centers for mentally and physically handicapped people, hot water tanks positive for legionellae were heated to 71°C for 72 hours followed by flushing for 15 minutes. In one center, legionellae reoccurred after 3 months. A quarterly heating schedule was established in both centers (Beam et al., 1984).

Ciesielski et al. (1984) demonstrated the importance of eliminating stagnation in hot water tanks in reducing the numbers of legionellae. Aerator screens in faucets were shown to provide niches for colonization by legionellae because they obstruct water flow.

Cordes et al. (1981) sterilized Legionella-contaminated showerheads with ethylene oxide but they were soon recontaminated.

Use of Ozone -- Ozone is, in general, a more potent disinfectant than chlorine but, like chlorine, it is subject to reduced disinfection efficiency at higher pH ranges (Engelbrecht, 1983). On the other hand, water temperature appears to have either no effect on disinfection efficiency or to increase efficiency. Turbidity may cause some reduction in the bacteriocidal efficiency of ozone, and the presence of organic matter certainly reduces efficiency (Engelbrecht, 1983). When a residual is established, the rate of inactivation is very rapid. If water is free of turbidity and organic matter, the conditions are optimal for effective disinfection with ozone. The major disadvantages are the difficulty of maintaining a residual in the distribution system and the expense of disinfection.

In 1977 ozone was only used in five water treatment facilities in the United States and in some 1039 facilities worldwide (Engelbrecht, 1983).

Edelstein et al. (1982d) used ozone in an attempt to eradicate legionellae from the potable water supply of an unused wing of a hospital that was known to be contaminated with the bacteria. The results were inconclusive because the organisms were eliminated from both the experimental wing and the control wing that was untreated. The latter was thought to be due to excess mechanical flushing and an unexpected rise in the chlorine content of the mains water supply. The in vitro susceptibility of L. pneumophila to ozone was on the order of 0.36 mg/ℓ but was not consistent. The ozone mean residual concentration used in the hospital water system was 0.79 mg/ℓ.

Use of Ultraviolet (UV) -- Antopol and Ellner (1979) reported that 90% of L. pneumophila cells in distilled water were killed by 920 μW-s/cm² of UV radiation. This could be compared with intensities ranging from 2100 to 5500 μW-s/cm² for killing of E. coli, Salmonella, Serratia, and Pseudomonas. If the latter values were obtained under the same conditions as those used for L. pneumophila, the LD bacteria may be more than twice as susceptible to UV radiation.

Gilpin (1984) reported laboratory and field experiments using UV to inactivate Legionella spp. in standing and recirculating water systems. Times of exposure to 1 μW/cm² of UV radiation to produce 90% killing of Legionella spp. ranged from 17-44 minutes. A commercial UV apparatus killed 99% of Legionella spp. in less than 30 seconds in a 3-liter recirculating water system.

Summary

The concentration of groups at high risk from LD are those in hospitals and other primary-care institutions. The major sources of legionellae to which these patients are exposed are heat-rejection equipment and internal water supplies. The latter consist of showers, faucets, toilets, respiratory therapy equipment, water holding tanks, hot water tanks, etc. Some hospitals, for example in England, have dual water supplies, potable and nonpotable.

Nosocomial pneumonia caused by legionellae is a serious disease because it usually affects debilitated and immunocompromised patients with little resistance to infection. When disease is occurring and the causative organism is present and epidemiologically associated with patients of known susceptibility, few would disagree that stringent measures of control are indicated. Some participants in the 2nd International Symposium on _Legionella_ believed that the mere presence of the organism in the potable water of an institution justified control procedures even in the absence of illness. This view is motivated to some extent by an aspect of legal liability. All agreed that legionellae in hospital respiratory equipment must be controlled. This can be done simply by using sterile tapwater. Humidifiers, particularly those of the spinning disc type, are potentially hazardous. Aerosols of _L. pneumophila_ in a stationary phase of growth and at an appropriate relative humidity may be viable and stable for periods of 2 hours or more.

Because many hospitals have neither performed surveillance for undiagnosed cases of LD nor for water sites contaminated with legionellae, an important disease problem may not be recognized. This has proved to be the case in some hospitals.

The two major methods for direct control of LDB in the hospital water system are chlorine and heat. If legionellae are controlled by heat, care must be taken to prevent scalding of patients. Results of chlorine disinfection are frequently erratic in hot water systems because of the difficulty of maintaining an appropriate residual. This is most effectively accomplished with continuous-flow proportional chlorinators that maintain free chlorine residuals of 1-2 mg/ℓ or more. At these elevated chlorine levels, corrosion of the distribution system may become a major problem. Successful operation depends upon close supervision of the system.

Prevention and control of legionellae in drinking water should include better management of storage reservoirs to prevent addition of organic matter and growth of algae and protozoa.

The entire water distribution system in new construction must be thoroughly flushed and disinfected before being put into operation. Storage tanks should be covered. Hot water tanks should be designed to give uniform temperatures throughout. Dead-end piping should be eliminated. Hot or cold water tanks used intermittently should be disconnected from the system, drained, flushed, and disinfected before being reconnected. Hot water tanks should be drained regularly or at least bled to remove accumulated sludge that serves as a substrate for growth of legionellae and other microorganisms. Taps and showers in unused areas should at least be flushed before patients are exposed to them. Sieves and aerators should be removed from faucets. Rubber washers and gaskets should be replaced with materials that do not support growth of legionellae.

No standard procedures exist for disinfection of potable water for elimination of legionellae. Water temperature to be maintained, disinfectant concentration and schedule to be chosen, and all other management techniques to be used have been tailored to specific problems. As an editorial writer in _Lancet_ put it, "Perhaps the simplest measure in addition to continuous disinfection is to insure that the hot water is indeed hot, and the cold water at least cool." Summarizing the ecology, transmission, and control of legionellosis at the 2nd International Symposium on _Legionella_, Seidler (1984) concluded that those most concerned with the problem of legionellosis think it unlikely that a standard procedure will soon become acceptable for control of legionellae in the environment.

In discussing sources of legionellosis at the same symposium, Fraser (1984) expressed an uneasy feeling that something is being missed - that not all important sources of _Legionella_ have been identified.

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